MITIGATING ANTIMICROBIAL RESISTANCE IN ANIMALS

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ABSTRACT

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An increasing number of widespread antimicrobial resistance (AMR) issues involving animals have been reported in the recent years. These have spurred strong skepticism and criticism on current practices in animal management which controversially often involves non-therapeutic applications of antimicrobials. This also highlighted the fact that prudent use of veterinary antimicrobials is a key component in mitigating the emergence and spread of AMR. This dissertation investigates some of the current AMR issues involving animals and explores potential solutions that may alleviate the implication of the animals and the veterinary profession in the emergence and spread of antimicrobial resistance. Chapter 1 is an overview of AMR principles, current issues, and public health impact. Chapter 2 investigates the extent of vancomycin resistant enterococci (VRE) in pigs in the United States. Chapters 3 and 4 cover the development and evaluation of Antimicrobial Resistance Learning Site (AMRLS), a web-based learning tool for Veterinary Students intended to enhance their understanding about AMR and their crucial role as future veterinarians in mitigating AMR through antimicrobial stewardship and supporting policies fostering its mitigation.

"I have fought the good fight, I have finished the race, I have kept the faith." (2 Tim 4:7)

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KEY TO SYMBOLS OR ABBREVIATIONS

AMR	Antimicrobial Resistance
AMRLS	Antimicrobial Resistance Learning Site
CC	Clonal Complex
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CLSI	Clinical Laboratory and Standards Institute
CVM, MSU	College of Veterinary Medicine, Michigan State University
FDA	Food and Drug Administration
HHS	Department of Health and Human Services
MLST	Multi-locus Sequence Typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
NARMS	National Antimicrobial Resistance Monitoring System
PBPs	Pencillin-binding proteins
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
ST	Sequence type
TPB	Theory of Planned Behavior
VRE	Vancomycin-resistant enterococci
VREf	Vancomycin-resistant *Enterococcus faecium*
VRSA	Vancomycin-resistant *Staphylococcus aureus*

INTRODUCTION

Since their introduction, antimicrobials have revolutionized man's approach to treatment, control and prevention of human and animal infectious diseases. The modern antibiotic era markedly improved survival rates and longevity as catastrophic disease outbreaks were controlled and previously fatal infections became clinically manageable. Overall, these changes greatly improved the quality of human life and animal welfare.

However, the emergence and spread of antimicrobial resistance has become as a major problem. This global phenomenon has raised the alarming possibility of subsequent generations returning to the pre-antibiotic era when common infections were often fatal due to the lack of effective treatments. Medical history and research has shown that the prevalence of resistant bacteria and resistant genes increase in response to the selective pressure created by the use of antibiotics. Evidence is mounting that much of the problem is rooted in the inappropriate and excessive use of antimicrobials, and that one of the most effective counter measures is to practice prudent and judicious antimicrobial usage. To achieve this societal change, we must empower health care professionals with the resources and information they need to facilitate sound decisions pertaining to antimicrobial usage.

The worldwide animal industry is estimated to use more tons of antibiotics than does human medicine. For the growing antimicrobial resistance problem to be effectively contained or reversed, responsible antimicrobial use in the human medical community must be accompanied by a corresponding effort among veterinarians and others in the food animal and companion

animal industries. Veterinarians should be leaders in the appropriate use of antimicrobial agents for their patients, and should also understand how the use of antimicrobial agents in animals may affect the health of humans. Veterinarians should also advise their clients regarding the appropriate use of antimicrobial agents purchased over the counter, and should engage in educational activities that acknowledge themselves as the health professionals that are best able to regulate and control the public's access to antibiotics used for animals.

CHAPTER 1

ANTIMICROBIAL RESISTANCE IN ANIMALS: AN OVERVIEW

Abstract

Antimicrobial resistance is the ability of a microorganism to survive and multiply in the presence of an antimicrobial agent that would normally inhibit or kill this species of microorganism. It is not a new phenomenon, but in the recent years the global increase in incidence and prevalence of antimicrobial resistance, moreso, multiple drug resistance, has raised concerns as this has resulted to limited therapeutic options for infections in both animals and people. Several epidemiological and molecular evidences have already shown that AMR, as fostered by extensive antibiotic usage in animals, can increase AMR problems among human populations. Veterinarians must thus recognize, understand and appreciate their roles and professional responsibility in preventing AMR to help mitigate this growing issue in both animal and public health.

1.1 Antimicrobial Resistance

The introduction of antimicrobials transformed human and animal health systems by revolutionizing our weaponry in the war against infectious diseases, resulting in improved survivability for both humans and their domestic animals. However, this health triumph was immediately ebbed by the subsequent realization that bacterial populations could quickly modify themselves to resist antimicrobials, propagate these resistance traits, and even share resistance genes with other contemporary bacteria within their environment. Such abilities have seriously

compromised the usefulness of antibiotics in the war against microbes and warn of a future when antimicrobials may have very limited usefulness to control bacterial infection

Antimicrobial resistance is the ability of a microorganism to survive and multiply in the presence of an antimicrobial agent that would normally inhibit or kill this particular kind of organism. Antimicrobial resistance is just one of the many adaptive traits that resilient bacterial subpopulations may possess or acquire, enabling them to out-compete and out-survive their microbial neighbors and overcome host strategies aimed against them. This phenomenon is nearly as old as the discovery of antimicrobials themselves, having been described by pioneers like Ehrlich for trypanosomes (32) and Fleming for staphylococci (41). What is most alarming today is the rate at which antibiotic resistance often develops and how quickly it spreads across the globe and among different species of bacteria.

Furthermore, as a result of sequential, cumulative acquisition of resistance traits against different antibiotics, more bacterial pathogens with **multiple-drug resistance** are being reported worldwide. As a consequence, many bacterial organisms, including major human and animal pathogens such as *Mycobacterium* and *Salmonella* species, have become resistant to antibiotics which were previously quite efficacious.

Resistance to single antibiotics became prominent in organisms that encountered the first commercially produced antibiotics. The most notable example is resistance to penicillin among staphylococci, specified by an enzyme (penicillinase) that degraded the antibiotic. Over the years, continued selective pressure by different drugs has resulted in organisms bearing

additional kinds of resistance mechanisms that led to multidrug resistance (MDR), novel penicillin-binding proteins (PBPs),enzymatic mechanisms of drug modification, mutated drug targets, enhanced efflux pump expression, and altered membrane permeability. Some of the most problematic MDR organisms that are encountered currently include *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella pneumoniae* bearing extended-spectrum β-lactamases (ESBL), vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant MRSA, and extensively drug-resistant (XDR) *Mycobacterium tuberculosis*. (7)

Table 1.1 Antibiotic Timeline

Time Period	Discovery and introduction	Emergence of resistance
Before 1930	Discovery of penicillin (1929)	-
1930 to 1940	Introduction of sulfonamide	Efficacy of penicillin in humans shown; sulfonamides introduced in food animal use
1941 to 1950	Introduction of streptomycin (1944), chloramphenicol (1946) and chlortetracycline (1948)	Penciillin made available to the public; widespread use in animals by 1950.
1951 to 1960	Introduction of erythromycin, vancomycin, tylosin and methicillin	Penicillin-resistant infections become clinically significant
1961 to 1970	Introduction of gentamicin (1963), ampicillin (1966), cephalothin (1966), amikacin (1970)	Emergence of gentamicin-resistant *Pseudomonas* (1968); emergence of methicillin-resistant staphylococcal infections (1968)
1971 to 1980	Introduction of carbenicillin (1973), cefoxitin (1978), cefaclor (1979)	Increasing trend of nosocomial infections due to opportunistic pathogens; Ampicillin-resistant infections become frequent
1981 to 1990	Introduction of cefotaxime (1981), clavulanic acid-amoxicillin (1983), imipenem-cilastatin (1985), norfloxacin (1986), aztreonam (1986)	Spread of methicillin-resistant staphylococcus infections; emergence of AIDS-related bacterial infections

Table 1.1 (cont'd)

1991 to 2000	Introduction of oral extended spectrum cephalosporins (1998), Quinupristin-dalfopristin (1999), linezolid	Emergence of vancomycin-resistant enterococci; emergence of multi-drug resistant *Mycobacterium tuberculosis;* global emergence of multi-drug resistant *Salmonella enteric* serovar Typhimurium DT 104
2001 to 2008	Introduction of broader spectrum fluoroquinolones (2001), Telithromycin (2002), Tigecycline (2006)	Emergence of vancomycin-resistant staphylococcal infections; Spread of extended-spectrum beta-lactamase among Gram negatives; Emergence of more multi-drug resistant organisms

1.2 Bacterial Resistance Strategies

To survive in the presence of an antibiotic, bacterial organisms must be able to disrupt one or more of the essential steps required for the effective action of the antimicrobial agent. The intended modes of action of antibiotics may be counter-acted by bacterial organisms via several different means. This may involve preventing antibiotic access into the bacterial cell or perhaps removal or even degradation of the active component of the antimicrobial agent. No single mechanism of resistance is considered responsible for the observed resistance in a bacterial organism. In fact, several different mechanisms may work together to confer resistance to a single antimicrobial agent. There are four major bacterial resistance strategies:

A. By prevention of the antimicrobial from reaching its target by reducing its ability to penetrate into the cell.

Antimicrobial compounds almost always require access into the bacterial cell to reach their target site where they can interfere with the normal function of the bacterial organism. Porin

channels are the passageways by which these antibiotics would normally cross the bacterial outer membrane. Some bacteria protect themselves by prohibiting these antimicrobial compounds from entering past their cell walls. For example, a variety of Gram-negative bacteria reduce the uptake of certain antibiotics, such as aminoglycosides and beta lactams, by modifying the cell membrane porin channel frequency, size, and selectivity. Prohibiting entry in this manner will prevent these antimicrobials from reaching their intended targets that, for aminoglycosides and beta lactams, are the ribosomes and the penicillin-binding proteins (PBPs), respectively.

This strategy have been observed in:*Pseudomonas aeruginosa* against imipenem (a beta-lactam antibiotic); *Enterobacter aerogenes* and *Klebsiella* spp. against imipenem; vancomycin intermediate-resistant *S. aureus* or VISA strains with thickened cell wall trapping vancomycin; many Gram-negative bacteria against aminoglycosides; and many Gram-negative bacteria against quinolones

B. By expulsion of the antimicrobial agents from the cell via general or specific efflux pumps.

To be effective, antimicrobial agents must also be present at a sufficiently high concentration within the bacterial cell. Some bacteria possess membrane proteins that act as an export or efflux pump for certain antimicrobials, extruding the antibiotic out of the cell as fast as it can enter. This results in low intracellular concentrations that are insufficient to elicit an effect. Some efflux pumps selectively extrude specific antibiotics such as macrolides, lincosamides, streptogramins and tetracyclines, whereas others (referred to as multiple drug resistance pumps) expel a variety of structurally diverse anti-infectives with different modes of action.

This strategy has been observed in: *E.coli* and other Enterobacteriaceae against tetracyclines; Enterobacteriaceae against chloramphenicol; Staphylococci against macrolides and streptogramins; *Staphylococcus aureus* and *Streptococcus pneumoniae* against fluoroquinolones;

These efflux pumps are variants of membrane pumps possessed by all bacteria, both pathogenic and non-pathogenic, to move lipophilic or amphipathic molecules in and out of the cells. Some are used by antibiotic producers to pump antibiotics out of the cells as fast as they are made, and so constitute an immunity protective mechanism for the bacteria to prevent being killed by their own chemical weapons. (110)

C. By inactivation of antimicrobial agents via modification or degradation.

Another means by which bacteria preserve themselves is by destroying the active component of the antimicrobial agent. A classic example is the hydrolytic deactivation of the beta-lactam ring in penicillins and cephalosporins by the bacterial enzyme called beta lactamase. The inactivated penicilloic acid will then be ineffective in binding to PBPs (penicllin binding proteins), thereby protecting the process of cell wall synthesis. This strategy has also been observed in: Enterobacteriaceae against chloramphenicol (acetylation); Gram negative and Gram positive bacteria against aminoglycosides (phosphorylation, adenylation, and acetylation).

The first antibiotic resistance mechanism described was that of penicillinase. Its presence and activity was first reported by Abraham and Chain in 1940 shortly after its discovery (5). Less

than 10 years after the clinical introduction of penicillins, penicillin-resistant *Staphylococcus aureus* was observed in a majority of Gram-positive infections in people. The initial response by the pharmaceutical industry was to develop beta-lactam antibiotics that were unaffected by the specific beta-lactamases secreted by *S. aureus*. However, as a result, bacterial strains producing beta-lactamases with different properties began to emerge, as well as those with other resistance mechanisms. This cycle of resistance counteracting resistance continues even today (16)

D. By modification of the antimicrobial target within the bacteria.

Some resistant bacteria evade antimicrobials by reprogramming or camouflaging critical target sites to avoid recognition. Therefore, in spite of the presence of an intact and active antimicrobial compound, no subsequent binding or inhibition will take place.

Examples of bacterial resistance due to target site modification include: alteration in penicillin-binding protein (PBPs) leading to reduced affinity of beta-lactam antibiotics (Methicillin-Resistant *Staphylococcus aureus, S. pneumoniae*, *Neisseria gonorrheae*, Group A streptococci, *Listeria monocytogenes*); changes in peptidoglycan layer and cell wall thickness resulting to reduced activity of vancomycin: Vancomycin-resistant S. *aureus* ; changes in vancomycin precursors reducing activity of vancomycin: *Enterococcus faecium* and *E. faecalis;* alterations in subunits of DNA gyrase reducing activity of fluoroquinolones in many Gram-negative bacteria; alteration in subunits of topoisomerase IV leading to reduced activity of fluoroquinolones: Many Gram positive bacteria, particularly *S.auerus* and *Streptococcus pneumoniae* ; changes in RNA polymerase leading to reduced activity of rifampicin: *Mycobacterium tuberculosis* (12, 71).

Table 1.2 Mechanisms of Resistance Against Different Antimicrobial Classes (12, 42)

ANTIMICROBIAL CLASS	MECHANISM OF RESISTANCE	SPECIFIC MEANS TO ACHIEVE RESISTANCE	EXAMPLES
Beta-lactams Examples: penicillin, ampicillin, mezlocillin, peperacillin, cefazolin, cefotaxime, ceftazidime, aztreonam, imipenem	Enzymatic destruction	**Destruction of beta-lactam rings by beta-lactamase enzymes.** With the beta-lactam ring destroyed, the antibiotic will no longer have the ability to bind to PBP (Penicillin-binding protein), and interfere with cell wall synthesis.	**Resistance of staphylococci to penicillin;** Resistance of *Enterobacteriaceae* to penicllins, cephalosporins, and aztreonam
	Altered target	**Changes in penicillin binding proteins.** Mutational changes in original PBPs or acquisition of different PBPs will lead to inability of the antibiotic to bind to the PBP and inhibit cell wall synthesis	Resistance of staphylococci to methicillin and oxacillin
	Decreased uptake	**Porin channel formation is decreased.** Since this is where beta-lactams cross the outer membrane to reach the PBP of Gram-negative bacteria, a change in the number or character of these channels can reduce betalactam uptake.	Resistance of *Enterobacter aerogenes, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* to imipenem
Glycopeptides Example: vancomycin	Altered target	**Alteration in the molecular structure of cell wall precursor components** decreases binding of vancomycin so that cell wall synthesis is able to continue.	Resistance of enterococci to vancomycin

Table 1.2 (cont'd)

Aminoglyosides Examples: gentamicin, tobramycin, amikacin, netilmicin, streptomycin, kanamycin	Enzymatic modification	**Modifying enzymes** alter various sites on the aminoglycoside molecule so that the ability of this drug to bind the ribosome and halt protein synthesis is greatly diminished or lost entirely.	Resistance of many Gram-positive and Gram negative bacteria to aminoglycosides
	Decreased uptake	**Change in number or character of porin channels** (through which aminoglycosides cross the outer membrane to reach the ribosomes of gram-negative bacteria) so that aminoglycoside uptake is diminished.	Resistance of a variety of Gram-negative bacteria to aminoglycosides
	Altered target	**Modification of ribosomal proteins or of 16s rRNA.** This reduces the ability of aminoglycoside to successfully bind and inhibit protein synthesis.	Resistance of *Mycobacterium* spp to streptomycin
Quinolones Examples: ciprofloxacin, levofloxacin, norfloxacin, lomefloxacin	Decreased uptake	**Alterations in the outer membrane** diminishes uptake of drug and/or activation of an "efflux" pump that removes quinolones before intracellular concentration is sufficient for inhibiting DNA metabolism.	Resistance of Gram negative and staphylococci (efflux mechanism only) to various quinolones
	Altered target	**Changes in DNA gyrase subunits** decrease the ability of quinolones to bind this enzyme and interfere with DNA processes	Gram negative and Gram positive resistance to various

1.3. Molecular mechanisms of resistance

The abilities of bacterial organisms to utilize the various strategies to resist antimicrobial compounds are all genetically encoded. Intrinsic resistance is that type of resistance which is naturally coded and expressed by all (or almost all) strains of that particular bacterial species. An example of instrinsic resistance is the natural resistance of anaerobes to aminoglycosides and Gram-negative bacteria against vancomycin.

Changes in bacterial genome through mutation or horizontal gene acquisition,on the other hand, may consequently lead to a change in the nature of proteins expressed by the organism. Such change may lead to an alteration in the structural and functional features of the bacteria involved, which may result in changes leading to resistance against a particular antibiotic. This is referred to as acquired resistance, which is limited to selected isolates of that particular species or group of microorganisms.

For example, we know that methicillin resistance of *Staphylococcus aureus* is primarily due to changes that occur in the penicillin binding protein (PBP), which is the protein which beta-lactam antibiotics bind and inactivate to consequently inhibit cell wall synthesis. This change is actually rendered by the expression of a certain *mec*A gene in some strains of these bacteria, which is hypothesized to have been induced by the excessive use of penicillin. Expression of this *mec*A gene results in an alternative PBP (PBP2a) that has a low affinity for most ß-lactam antibiotics, thereby allowing these strains to replicate in the presence of methicillin and related antibiotics.

Some antimicrobial resistance is brought about by multiple changes in the bacterial genome. For example, Isoniazid resistance of *Mycobacterium tuberculosis* results from changes in the following genes: *kat*G gene which encodes a catalase; *inh*A gene which is the target for isoniazid; the *oxy*R gene and neighboring *aph*C gene and their intergenic region.

Biological resistance refers to changes that result in the organism being less susceptible to a particular antimicrobial agent than has been previously observed. When antimicrobial susceptibility has been lost to such an extent that the drug is no longer effective for clinical use, the organism is then said to have achieved clinical resistance. It is important to note that often, biologic resistance and clinical resistance do not necessarily coincide. From a clinical laboratory and public health perspective it is important to realize that biologic development of antimicrobial resistance is an ongoing process, while clinical resistance is dependent on current laboratory methods and established cut-offs. Our inability to reliably detect all these processes with current laboratory procedures and criteria should not be perceived as evidence that they are not occurring (42).

A. Intrinsic Resistance

Intrinsic resistance is the innate ability of a bacterial species to resist activity of a particular antimicrobial agent through its inherent structural or functional characteristics, which allow tolerance of a particular drug or antimicrobial class. This can also be called "insensitivity" since it occurs in organisms that have never been susceptible to that particular drug. Such natural insensitivity can be due to: lack of affinity of the drug for the bacterial target, inaccessibility of

the drug into the bacterial cell, extrusion of the drug by chromosomally encoded active exporters, innate production of enzymes that inactivate the drug.

Table 1.3 Examples of intrinsic resistance and their respective mechanisms (42, 48)

ORGANISMS	NATURAL RESISTANCE AGAINST:	MECHANISM
Anaerobic bacteria	Aminoglycosides	Lack of oxidative metabolism to drive uptake of aminoglycosides
Aerobic bacteria	Metronidazole	Inability to anaerobically reduce drug to its active form
Gram-positive bacteria	Aztreonam (a beta-lactam)	Lack of penicillin binding proteins (PBPs) that bind and are inhibited by this beta lactam antibiotic
Gram-negative bacteria	Vancomycin	Lack of uptake resulting from inability of vancomycin to penetrate outer membrane
Klebsiella spp.	Ampicillin (a beta-lactam)	Production of enzymes (beta-lactamases) that destroy ampicillin before the drug can reach the PBP targets
Stenotrophomonas maltophila	Imipenem (a beta-lactam)	Production of enzymes (beta lactamases) that destroy imipenem before the drug can reach the PBP targets.
Lactobacilli and *Leuconostoc*	Vancomycin	Lack of appropriate cell wall precursor target to allow vancomycin to bind and inhibit cell wall synthesis
Pseudomonas aeruginosa	Sulfonamides, trimethoprim, tetracycline, or chloramphenicol	Lack of uptake resulting from inability of antibiotics to achieve effective intracellular concentrations
Enterococci	Aminoglycosides	Lack of sufficient oxidative metabolism to drive uptake of aminoglycosides
	All cephalosporins	Lack of PBPs that effectively bind and are inhibited by these beta lactam antibiotics

Knowledge of the intrinsic resistance of a pathogen of concern is important in practice to avoid inappropriate and ineffective therapies. For bacterial pathogens which are naturally insensitive to a large number of classes of antimicrobials, such as *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa*, this consideration can pose a limitation in the range of options for treatment and thus consequently further increase the risk for emergence of acquired resistance.

B. Acquired Resistance

Acquired resistance is said to occur when a particular microorganism obtains the ability to resist the activity of a particular antimicrobial agent to which it was previously susceptible. This can result from the mutation of genes involved in normal physiological processes and cellular structures, from the acquisition of foreign resistance genes or from a combination of these two mechanisms.

Unlike intrinsic resistance, traits associated with acquired resistance are found only in some strains or subpopulations of each particular bacterial species. Laboratory methods are therefore needed to detect acquired resistance in bacterial species that are not intrinsically resistant. These same methods are used for monitoring rates of acquired resistance as a means of combating the emergence and spread of acquired resistance traits in pathogenic and non-pathogenic bacterial species. Acquired resistance results from successful gene change and/or exchange that may involve: mutation or horizontal gene transfer via transformation, transduction or conjugation.

Table 1.4 Examples of acquired resistance through mutation and horizontal gene transfer

ACQUIRED RESISTANCE THROUGH:	RESISTANCE OBSERVED	MECHANISM INVOLVED
Mutations	*Mycobacterium tuberculosis* resistance to rifamycins	Point mutations in the rifampin-binding region of *rpoB*
	Resistance of many clinical isolates to luoroquinolones	Predominantly mutation of the quinolone-resistance-determining-regiont (QRDR) of GyrA and ParC/GrlA
	E.coli, Hemophilius influenzae resistance to trimethoprim	Mutations in the chromosomal gene specifying dihydrofolate reductase
Horizontal gene transfer	*Staphylococcus aureus* resistance to methicillin (MRSA)	Via acquisition of mecA genes which is on a mobile genetic element called "staphylococcal cassette chromosome" (SCCmec) which codes for penicllin binding proteins (PBPs) that are not sensitive to ß-lactam inhibition
	Resistance of many pathogenic bacteria against sulfonamides	Mediated by the horizontal transfer of foreign *fol*P genes or parts of it
	Enterococcus faecium and *E. faecalis* resistance to vancomycin	Via acquisition of one of two related gene clusters VanA and Van B, which code for enzymes that modify peptidoglycan precursor, reducing affinity to vancomycin.

i. Mutation

A mutation is a spontaneous change in the DNA sequence within the gene that may lead to a change in the trait which it codes for. Any change in a single base pair may lead to a corresponding change in one or more of the amino acids for which it codes, which can then change the enzyme or cell structure that consequently changes the affinity or effective activity of the targeted antimicrobials.

In prokaryotic genomes, mutations frequently occur due to base changes caused by exogenous

agents, DNA polymerase errors, deletions, insertions and duplications. For prokaryotes, there is a constant rate of spontaneous mutation of about 0.0033 mutations per DNA replication that is relatively uniform for a diverse spectrum of organisms. The mutation rate for individual genes varies significantly among and within genes (50)

ii. Horizontal Gene Transfer

Horizontal gene transfer, or the process of swapping genetic material between neighboring "contemporary" bacteria, is another means by which resistance can be acquired. Many of the antibiotic resistance genes are carried on plasmids, transposons or integrons that can act as vectors that transfer these genes to other members of the same bacterial species, as well as to bacteria in another genus or species. Horizontal gene transfer may occur via three main mechanisms: transformation, transduction or conjugation.

Transformation involves uptake of short fragments of naked DNA by naturally transformable bacteria. Transduction involves transfer of DNA from one bacterium into another via bacteriophages. Conjugation involves transfer of DNA via sexual pilus and requires cell –to-cell contact. DNA fragments that contain resistance genes from resistant donors can then make previously susceptible bacteria express resistance as coded by these newly acquired resistance genes.

1.4 Veterinary Public Health And Antimicrobial Resistance

The control and prevention of AMR is becoming a public health priority as reports of AMR emergence and spread increase from around the world. Veterinarians are medical professionals,

and have a public health responsibility to ensure that antimicrobials are used appropriately and prudently to preserve the efficacy of antibiotics for both animals and humans. The bottom line is that we do not want our grandchildren to suffer the ill effects of antibiotic treatment failure because we squandered the efficacy of antibiotics when good alternative options were only slightly less convenient. Cost-benefit analysis of antimicrobial use policy must consider future costs as well as present costs.

A. Prudent Use Of Antimicrobials In Animals

Prudent use of antimicrobials, which is also referred to as "judicious use" or "antimicrobial stewardship", is the optimal selection of drug, dose and duration of antimicrobial treatment, along with reduction of the inappropriate and excessive use as a means of slowing the emergence of antimicrobial resistance (49).

Although this may be more straightforward for human medicine, the nature by which antimicrobials are utilized in animals and the influences of various stakeholders in the standards by which these are raised, make such practice more complicated for veterinary medicine. The prudent use of antimicrobials in veterinary medicine are principled guidelines created to prevent abusive use of antimicrobials in animals, primarily to curb or mitigate the imminent risk of breeding resistant microorganisms unresponsive to currently available chemotherapy in both animals and humans. Veterinarians are on the forefront of upholding such manner of use having dual roles of protecting animals from pain and suffering, while safeguarding the interest of the public health.

B. Animals, Humans And Antimicrobials.

Epidemiological and molecular observations have shown that AMR, as fostered by extensive antibiotic usage in animals, can increase AMR problems among human populations. For example, vancomycin resistant enterococci (VRE) in both animals and people have become prevalent in countries that used a glycopeptide growth promotant called avoparcin, which is structurally similar to vancomycin. Vancomycin is a very important antibiotic in human medicine that is often used a last line of defense for several types of infectious agents. Consequent discontinuation of avoparcin's use in animals was followed by a rapid subsequent decline in the incidence of VRE in both human and animal populations. However, VRE in Europe has not disappeared.

Genes encoding resistance to antibiotics used only for animals have been found in increasing prevalence among animal pathogens, in the commensal flora of humans, in zoonotic pathogens like *Salmonella* and in strictly human pathogens like *Shigella*. This indicates the clonal spread of resistant strains and the shared transfer of resistance genes among bacteria infecting both humans and animals (14).

The introduction of enrofloxacin in veterinary medicine was quickly followed by the emergence of fluoroquinolone resistance among *Campylobacter* isolates from broilers, and in humans shortly thereafter. As was the case with avoparcin, resistance to fluoroquinolones in human and

animal populations remained rare in countries that had not used fluoroquinolones in food animals (1).

An increase in AMR to third-generation cephalosporins in *Salmonella* and *E.coli* was also observed following the increased usage of these antibiotics in animals. Furthermore, its withdrawal and re-introduction were subsequently followed by a decline and resurgence, respectively, in AMR among animal and human *Salmonella* isolates.

C. Examples Of Important Antimicrobials In Humans Used In Animals For Treatment, Metaphylaxis Or Growth Promotion (47, 84)

Because a wide array of antimicrobials important for animal health and production are also important for preserving human health, use of these antibiotics in animal populations may negatively impact human health. While all AMR is a potential human health hazard, the preserved efficacy of some antibiotics is more critical to human health. Below is a list of antimicrobials used in both animals and humans, classified by the World Health Organization (WHO) according to their importance to human health.

Table 1.5 List of antimicrobials classified by the WHO as critically important for humans

Antibiotic classes	**ANIMALS**				**Humans**
	Species	**Disease treatment**	**Disease Prevention**	**Growth promotion**	
Aminoclycosides: gentamicin, neomycin, streptomycin	Beef cattle, goats, poultry, sheep, swine, certain plants	Yes	Yes	-	Yes

Table 1.5 (cont'd)

Penicillins: amoxicillin, ampicillin	Beef cattle, dairy cows, fowl, poultry, sheep, swine	Yes	Yes	Yes	Yes
Cephalosporins, third generation: ceftiofur	Beef cattle, dairy cows, poultry, sheep, swine	Yes	Yes	-	Yes
Glycopeptides: Avoparcin, vancomycin	Poultry, swine			Yes	Yes
Macrolides: erythromycin, tilmicosin, tylosin	Beef cattle, poultry, swine	Yes	Yes	Yes	Yes
Quinolones: (fluoroquinolones) sarafloxacin, enrofloxaxin	Beef cattle, poultry, swine	Yes	Yes	-	Yes
Streptogramins: Virginiamycin, quinupristin-dalfopristin	Beef cattle, poultry, swine	Yes	Yes	Yes	Yes
Carbapenems, lipopeptides, oxazolidinones, cycloserine, ethambutol, ethionamide, isoniazid, para-aminosalicyclic acid, pyrazinamide		-	-	No	Yes

Table 1.6 List of antimicrobials classified by the WHO as highly important for humans

Antibiotic classes	ANIMALS				Humans
	Species	**Disease treatment**	**Disease Prevention**	**Growth promotion**	
Cephalosporins, first generation: cefadroxil	-	-	-	-	Yes
Cephalosporins, second generation: cefuroxime	-	-	-	-	Yes
Spectinomycin	Poultry, swine		Yes		Yes
Sulfonamides: sulfadimethoxine, sulfamethazine, sulfisoxazole	Beef cattle, dairy cows, fowl, poultry, swine, catfish, trout, salmon	Yes	-	Yes	Yes
Tetracyclines: Chlortetracycline, oxytetracycline, tetracycline	Beef cattle, dairy cows, honey bees, poultry, sheep, swine, catfish, trout, salmon, lobster	Yes	Yes	Yes	Yes
Cephamycins, dofazimine, monobactams, amino-penicillins, antipseudomonal penicillins, sulfones	-	-	-	-	Yes

Table 1.7 List of antimicrobials classified by the WHO as important for humans

Antibiotic classes	ANIMALS				Humans
	Species	**Disease treatment**	**Disease Prevention**	**Growth promotion**	
Polypeptides: Bacitracin	Fowl, poultry, swine	Yes	Yes	Yes	Yes
Lincosamides: Lincomycin	Poultry, swine	Yes	Yes	-	Yes

Table 1.8 List of antimicrobials not known to be used in humans

Antibiotic classes	ANIMALS				Humans
	Species	**Disease treatment**	**Disease Prevention**	**Growth promotion**	
Babermycin: Flavomycin	Beef cattle, poultry, swine		Yes	Yes	
Ionophores: monensin, salinomycin, semduramicin, lasalocid	Beef cattle, fowl, goats, poultry, rabbits, sheep		Yes	Yes	

1.5 The Human Health Impact Of Antimicrobial Resistance In Animal Populations

Animal production practices have evolved over the years to meet the food protein needs of the growing human population. Some farms became very large, and used modern production practices to push food animal growth rates to their optimum. Disease prevention, husbandry, genetics and nutrition have greatly improved the efficiency of many food animal production facilities.

To some degree, the industrialization of animal production was made possible by the availability of antibiotics for livestock and poultry. Although antibiotic usage has clearly benefited the animal industry and helped provide affordable animal protein to the growing human population, the use of antibiotics in food production also contributed to the emergence and spread of AMR. Along with antibiotics used for human medicine, antibiotics used for animal treatment, prophylaxis and growth promotion exerts an inestimable degree of selective pressure toward the emergence and propagation of resistant bacterial strains.

Antibiotic usage in veterinary practice may impact human health because animals can serve as mediators, reservoirs and disseminators of resistant strains and/or AMR genes. Consequently, imprudent use of antimicrobials in animals may unnecessarily result in increased human morbidity, increased human mortality, reduced efficacy of related antibiotics used for human medicine, increased healthcare costs, increased potential for carriage and dissemination of pathogens within human populations and facilitated emergence of resistant human pathogens.

A. Increased human morbidity

Due to their enhanced survivability in the presence of antibiotic concentrations, infectious agents possessing AMR traits gain an enhanced potential for transmission, incidence and persistence. This can result in their dominance over the prevailing microflora within mammalian host populations, leading to higher rates of transmission as compared to the susceptible bacterial strains. This is particularly important for zoonotic agents present in animal carriers in which the bacteria have gained the ability to resist antibiotics important for their treatment, control and prevention. Their enhanced ability to survive, thrive, prevail and resist treatment allows these resistant bacteria to be carried and maintained in their host animals, and therefore facilitates their spread to other susceptible hosts, including humans.

An example is the increasing frequency of quinolone resistance among *Salmonella* Enteritidis (84) and *Campylobacter spp* isolated from animals and people (9, 52), and the multiple resistance of *Salmonella* Typhimurium for ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (ACSSuT) (83).

Although resistance in strictly human pathogens such as *Shigella* spp. and *Salmonella typhi* is primarily attributed to the use of antibiotic agents in human populations, the use of antibiotics in agriculture is thought to be the principal driver of increasing resistance for many enteric zoonotic infectious agents for which animal populations serve as the principal epidemiological reservoir. The Department of Health and Human Services (HHS), Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) believe that resistant strains of three major bacterial pathogens in humans – *Salmonella*, *Campylobacter* and *E. coli* - are linked to the use of antibiotics in foodborne animals. These organisms are three of the top five major foodborne agents that account for an estimated 90% of deaths resulting from infection with foodborne pathogen in the United States (80).

The emergence of fluoroquinolone resistance among domestically acquired human infections with *Campylobacter jejuni* and *E. coli* is an example of AMR thought to have resulted from the use of antimicrobial agents in food animals and subsequent transmission of resistant bacteria to humans via the food supply Both molecular and epidemiological evidence indicate that the resulting AMR prevalence among humans was triggered by the introduction of enrofloxacin in poultry, prompting FDA to withdraw its approval for use in poultry in 2005 (36).

B. Increased human mortality

Higher case fatality rates are seen for patients infected with AMR organisms compared with those infected with antibiotic sensitive organisms (57). Physicians rely on empirical antibiotic treatments when therapy is urgent and cannot wait for laboratory testing, but empirical

treatments may fail when the pathogen has gained resistance. Empirical treatments are experience-based, therapeutic regimens generally administered prior to confirmatory diagnosis Examples are the failure of quinolones in treating invasive salmonellosis or the failure of vancomycin in managing infection with nosocomial vancomycin-resistant enterococci (VRE).

While some antibiotics are used empirically as the "first line of defense", other more toxic, more expensive or narrow spectrum antibiotics are reserved for use as the "last line of defense" against infections due to resistant pathogens. However, resistance to even the newest and most expensive "last defense" antibiotics has now been documented., e.g. vancomycin failure in treating for methicillin-resistant *Staphylococcus aureus* (MRSA). Additionally, the acquisition of AMR traits by some pathogens may be accompanied by additional pathogenicity and virulence genetic factors that increase the probability of patient death.

Helms *et al.* (55) found that patients infected with pansusceptible *Salmonella* Typhimurium were 2.3 times more likely to die within 2 years after infection than persons in the general Danish population, and that patients infected with strains resistant to ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline were 4.8 times (95% CI 2.2 to 10.2) more likely to die within 2 years. Furthermore, they established that quinolone resistance in this organism was associated with a mortality rate 10.3 times higher than the general population (55). Evidence is also mounting that, for some pathogens, increases in virulence often accompany acquisition of resistance.

The impact of AMR to the older and cheaper antibiotics is probably greater in developing countries where more expensive treatment alternatives are unavailable or unaffordable. It is impossible to quantify the increased human morbidity and mortality occurring in developing countries due to treatment failure with older antibiotics such as tetracyclines and penicillins that may be the only antibiotics available to people living in poverty.

C. Reduced efficacy to related antibiotics used in human medicine

Antimicrobial resistance due to a particular antibiotic used in food animals may result in reduced efficacy of most or all members of that same antibiotic class, some of which may be extremely important for human medicine. This occurs because of the similarity of the antibiotic's related structural components, which causes cross-recognition and cross-resistance for all or most of the antibiotics within the same antibiotic class. An example is the emergence and spread of vancomycin resistant enterococci (VRE) in hospitals following the extensive use of avoparcin in animals, a glycopeptide antimicrobial agent that is structurally similar to vancomycin. Another example is virginiamycin resistance cross-reacting with resistance to the human streptogramin, quinupristin-dalfopristin (77).

Streptogramins were developed for use in animals at a time when there was no interest in using this class of antibiotics for human medicine. Virginiamycin had been used subtherapeutically for growth promotion in livestock and poultry since 1974, However, after using virginiamycin in animals for many decades, researchers went back and re-visited the streptogramin class of antibiotics and developed quinupristin-dalfopristin for human usage. It was very disheartening

in 1999 when this newly licensed human antibiotic was immediately met with AMR to *Enterococcus faecium* due to many years of using virginiamycin in animals.

Enterococci are members of the normal gut flora for most warm-blooded animals, including humans. However, they are sometimes problematic nosocomial infections in hospital settings where the use of antibiotics is believed to contribute to the emergence of multiple antibiotic resistant genes in this organism. Vancomycin is considered the treatment of choice for many resistant organisms, so the emergence and subsequent spread of VRE became a significant public health concern.

Before the 1990s, it was thought that VRE were present only in hospitals where vancomycin had been used for many years (112). However, epidemiological and molecular studies have shown that the use of avoparcin in farm animals can result in carriage and dissemination of VRE by these animals and in humans in close contact with these animals (75, 112). Because of public health concerns about resistance to these glycopeptide antibiotics, avoparcin was banned in Denmark in 1995, in Germany in 1996, and eventually by all EU member states (112). Subsequent reduction in prevalence of VRE in poultry, swine, and humans in the later years were reported (111). Although vancomycin is frequently used in the hospital setting in the USA, avoparcin was never used in livestock and poultry in the US. This may be the reason why, in spite of the relatively high rates of VRE in U.S. hospitals, there is less evidence of a community reservoir for VRE in this country (92).

D. Increased human healthcare costs

An increased healthcare cost is another important consequence of antimicrobial resistance. Increased costs may be due to the need for additional antibiotic treatments, longer hospitalization, more diagnostic tests, higher professional costs and more pain management. In 1998, the Institute of Medicine estimated the annual cost of infections caused by antibiotic-resistant bacteria to be US$ .4 to 5 million (79). With the increase in incidence and prevalence of AMR in the last few years, the current actual cost is now likely to be much higher. Again, increased health costs have more profound repercussions in poorer countries where resources are more limited and the lost efficacy of the older, lower-cost antibiotics is a more significant determinant of human morbidity and mortality.

E. Increased carriage and dissemination

Because of their survival advantage, resistant bacteria may remain viable for longer periods in the environment and in animal reservoirs where they can eventually be transmitted to humans. Acquisition of resistant bacteria from farm animals has been shown to occur either via ingestion of foods of animal origin (102) or via direct contact with infected animals (20, 56).

MRSA, for example, was first reported in 1961 and emerged as a sporadic problem in US hospitals. By the 1990s, MRSA was recognized as a serious worldwide nosocomial infection. MRSA strains are resistant to beta-lactam antibiotics, including those that are not affected by penicillinase. The resistance is mediated by a *mec*A gene which codes for a penicillin-binding protein (PBP2a) that has low affinity for beta-lactam antibiotics. In the last few years, animals

have been implicated in the maintenance, spread and transmission of some types of MRSA among humans. There is evidence that transmission of MRSA strains can occur from animals to humans, and vice-versa. MRSA has been found in humans closely associated with carrier animals; among pet owners (62), veterinarians and veterinary personnel (8, 118, 119) as well as pig and cattle farmers (69, 107). Studies identified both livestock and companion animals as potential sources of MRSA for humans, and close contact with these animals was identified as a risk factor for their carriage in people.

F. Facilitated emergence of resistance in human pathogens

Using mathematical models, Smith[29] demonstrated that the use of animal agricultural antibiotics can hasten the appearance of AMR bacteria in humans, with the greatest impact occurring soon after the first emergence of resistance. Although it is true that such changes and adaptations can occur independently of antimicrobial use in animals, the existence of resistance genes in animal populations can expedite the process by contributing a pool of resistant genes and resistant bacteria in the environment and reservoir hosts. This phenomena is illustrated in the resistance gene cycle depicted by Davies (24) which shows that resistance gene acquisition by various microorganisms could contribute to the environmental antibiotic resistance gene pool which then become a source of resistance genes for other types of bacteria.

For foodborne pathogens, the gastrointestinal tract has been the most important environment for gene transfer. Referred to as "The Reservoir Hypothesis", many believe that numerous species of intestinal bacteria have a significant role in storing and transmitting AMR genes. Several

authors have also reported transfer of genes in the rumen, in foodstuffs and in biofilms present on food processing equipment (67). Acquisition of resistance genes via conjugation or transformation in these environments may pose a serious health issue when a pathogen acquires resistance genes from the surrounding flora in the gastrointestinal tract.

Several findings *in vitro* and *in vivo* have demonstrated the occurrence of gene transfer in the alimentary tract. For example, tetracylcine and erythromycin genes encoded on transposons were shown to be transferable from *Enterococcus faecalis* to *E. coli* and *L. monocytogenes* in the digestive tract of mice (29). An epidemic R plasmid from *Salmonella enteritidis* moving to *Escherichia coli* of the normal human gut flora has also been observed. Several epidemiologic and molecular studies involving antimicrobial resistance of human and animal pathogens also support this hypothesis.

1.6. The environmental impact of imprudent antimicrobial use in animals

Another area of human health concern is the effect of antibiotic residues in the environment. Although human antimicrobial usage may be the primary source for aquatic and terrestrial antibiotic contamination, antibiotic applications in livestock, poultry and aquaculture also contribute significantly to this growing problem.

A varying proportion of administered antibiotics may remain active in excreted biological matter (generally feces or urine) after passing through the animal. Along with antimicrobials used for humans, the livestock, poultry and aquaculture sectors are important contributors to aquatic and

terrestrial contamination with antibiotics. Antibiotics and their metabolites (degradation products) reach the environment via the application of antibiotic-laden manure or slurry on agricultural lands, or direct deposition of manure by grazing animals. This can be followed by surface run-off, driftage or leaching into deeper layers of the earth (68). A proportion of the antibiotics that reach the environment will remain biologically active. Low subtherapeutic concentrations of antibiotics that accumulate over time may have profound effects on some ecosystems. Environmental antibiotic concentrations may exert selective pressure on environmental bacteria and may also foster the transfer of resistance genes, helping create the "resistome" mixing pot.

A. Veterinary antibiotics in soil

The concentration of antibiotics in various soil layers is termed "*terracumulation*" (91). Terracumulation will occur if an antibiotic is deposited in the soil at a rate that exceeds the rate of degradation. Antibiotics administered to animals are not completely absorbed by the animals to which they are administered. Depending on the antibiotic, 30-90% of the antibiotic can be excreted via urine or feces as intact bioactive substances or as antibiotic metabolites that may still have some antimicrobial activity. The excretion rate varies greatly, and depends on the pharmacokinetics of the administered antimicrobial, the route of application and the animal species involved. Antibiotics can also reach the soil through medical wastes, improper drug disposal or via dust from pens or barns. A growing number of studies worldwide provide evidence of the presence of many of veterinary antibiotics in the soil at concentrations reaching as high as 9,990ug kg^{-1}. Examples include: oxytetracycline and sulfachlorpyridazine (66), sulfamethazine and chlortetracycline (10).

Excreted compounds can be adsorbed, leached, degraded (through biotic or abiotic processes) and in some cases may revert back to the parent compound (93). Degradation in soil is mainly from microbial action on the antibiotic. Although antimicrobials may remain in the upper layer of the soil, sorptive affinity and other properties of the antibiotic and soil may cause the antibiotic to reach the groundwater layer.

Once in the environment, any continued antibiotic efficacy depends on its physical-chemical properties (molecular structure, size, shape, solubility and hydrophobicity), prevailing climatic conditions, soil types and other environmental factors (68). Antibiotic potency is mostly decreased by dilution, sorption and fixation, but antimicrobial activity may persist for long periods of time (98). No one answer is correct for all types of antibiotics.

B. Veterinary antibiotics in water

Contamination of the soil may be followed by surface run-off, driftage or leaching into the surface and/or the ground water. Also, antibiotics used for aquaculture may directly affect the aquatic environment, particularly when pens are placed in natural seawaters (99).

Antibiotics that have been reported in ground and surface water include macrolides, sulfonamides, tetracycline, chloramphenicol, chlortetracycline, sulfamethazine, lincomycin, trimethoprim, sulfadimethoxine and sulfamethazine. The veterinary and human antibiotic sulfamethoxazole was found in 23% of the 47 groundwater sites tested across the United States, and is one of the most frequently detected chemical compounds as determined by a national

survey of wastewater contaminants. A large proportion of aquatic antibiotic contamination is thought to be from human antibiotic usage, i.e. hospital effluents and municipal sewage and wastewater that eventually ends up in the environment (70).

C. Effects on other ecosystems

Veterinary antibiotics are designed to affect bacterial pathogens found in animals and people, but they certainly can also be hazardous to many types of non-targeted environmental microorganisms (76). High "therapeutic" concentrations of antibiotics tend to be quickly lethal to susceptible bacterial strains, providing limited opportunity for selection of subpopulations that have low or intermediate resistant traits. In contrast, low-level antibiotic concentration in soil and water may be more likely to lead to the selection of resistant environmental microorganisms fueling the environmental resistant gene pool or "resistome".

The overall ecologic impacts of residual antibiotics in the environment are largely unknown. However, antibiotics have been reported to markedly affect plant growth and development, causing inhibition of germination, inhibition of root growth and inhibition of shoot growth (15). It has also been shown to exhibit toxic effects to aquatic organisms such as freshwater crustacean *Daphnia magna* (117) and *Artemia spp*.(82).

1.7. Antimicrobial resistance: a global problem

Antibiotic resistance was initially viewed as only being a human medical problem in hospital-acquired infections, and usually only in critically ill and immunosuppressed patients. Today, the

AMR phenomena has spread to the point that the general population is considered to be at risk, bringing about an era where many common infections are becoming increasingly difficult to treat. One of the significant contributing factors to this changing trend is the spillover of AMR from excessive and poor stewardship of antibiotics in poultry and livestock.

The AMR phenomenon has become a global concern as geographic borders among countries and continents have become less distinct due to increasing global trade, expanding human and animal populations, societal advances and technological developments. Because of this increasing global connectivity, we now see rapid transport of infectious agents and their AMR genes. This means that AMR, in any obscure microscopic niche anywhere in the world, may consequently exert an impact on the rest of the world.

A. Veterinary-related Factors Influencing the Global Spread of AMR

i. Increase in population, demand for food animal protein and global changes in animal production systems.

The Center for Strategic and International Studies estimates that the world population increases by about 8,700 people every hour, 146 people every minute or 2.5 people every second. From 1950 to the year 2000, the population roughly doubled from 3 billion to 6.3 billion and is projected to continue to increase in the years to come (101).

Understandably, food production must also increase to meet these increased nutritional demands. However, because of urbanization and industrialization, available agricultural lands

continue to shrink and livestock production has become compromised (35) in many regions, including the EU (101).

In reaction to the increasing demand for food and the decreasing available agricultural land, most livestock and poultry are now raised in smaller spaces at the least possible cost and pushed to the fastest possible rate of gain. This often requires reliance on antibiotics for treatment, metaphylaxis or growth promotion; thereby creating concomitant increased rates of AMR.

ii. Changing trends in animal trading and increased movement of animals and animal byproducts.

The international trade in livestock and livestock products is a growing business, accounting for about one sixth, by value, of all agricultural trade (35). To liberalize international trade, the General Agreement for Tariffs and Trade (GATT) was established in 1947. Recognizing that animal health and food safety standards can be nontariff barriers to international free trade, the World Trade Organization (WTO) also incepted Sanitary and Phytosanitary (SPS) measures. The Office International des Epizooties (OIE) was tasked to set appropriate global standards for animal health, while the Codex Alimentarius Commission sets standards for food safety (4).

These standards facilitated safer international movement of animals and animal by-products around the world. However, they do little to prevent the spread of AMR across the globe due to resistant bacterial organisms that may be hitchhiking in animal products and healthy animals.

Increased movement of animals and animal by-products has also been facilitated by technological improvements in travel and transport systems. It used to be that food products with short shelf lives could not be moved to distant markets, but what used to take weeks and months to transport can now be moved within a day or even less. This rapid movement increases the likelihood that bacteria will remain viable while in transit, further increasing the risk that AMR genes can quickly spread around the world.

iii. Lack of Global Initiative Regarding AMR

In many countries there is little surveillance information regarding rates of antimicrobial usage or AMR in food or food animals. Such programs are expensive, and may also require a strong political will to counter the influence of some in the private sector who may not want information revealed that might scare consumers, jeopardize pharmaceutical sales or negatively affect exports or imports. Also, many countries have much more pressing issues such as feeding their people, fighting wars and developing their economies.

B. National and International AMR Programs

Today, AMR is no longer considered an unusual phenomenon as it was when first observed in the 1950's. Many national and international agencies are taking action to mitigate AMR and keep antibiotics effectively working to maintain the health of human and animal populations.

i. Monitoring antibiotic usage

Denmark has become an international leader in the fight against AMR. Antibiotic sales for humans and animals are monitored annually, as are rates of AMR in bacteria from food animals, food and people by the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP). The component that monitors antibiotic usage in veterinary practice is VetStat, which collects data from pharmacies, veterinarians and feed mills (103).

In the U.S. and many other countries, pharmaceutical companies are not required to report information regarding antibiotic sales. There are published approximations of antibiotic sales in the U.S., however these estimates differ greatly. The Union of Concerned Scientists estimated contemporary non-therapeutic usage of antimicrobials in cattle, swine and poultry at 24.6 million pounds (cattle: 3.7 million pounds; swine: 10.3 million pounds; poultry: 10.5 million pounds), basing their calculations from the number of animals, recommended uses and dosage. The Animal Health Institute's 2000 report estimated that antimicrobials used for growth promotion was at about 3.1 million pounds, with 14.7 million attributed to therapeutic use and disease prevention (81).

However, monitoring the total pounds of antibiotics used per year encourages us to equate the AMR pressure from all types of antibiotics, whereas it is much more important to conserve the efficacy of those antibiotics that are most important for human health. For example, the impact of a pound of tetracycline should in no way be equated with the impact of a pound of 3rd generation cephalosporin or fluoroquinolone.

A review by Sarmah (93) summarized a list of animal antibiotics registered for use as growth promoters and/or feed efficiency in Australia, European Union (EU), Canada and the USA (Table 1.9).

Table 1.9 Animal antibiotics registered for use as growth promoters/feed efficiency in Australia, EU, Canada, and the USA(93)

ANTIBIOTIC GROUP	COUNTRIES USING	ANTIBIOTIC	USAGE
Arsenicals	Australia	3-Nitro-arsonic acid	Pigs, poultry
	USA	Arsenilic acid, Roxarsone, cabarsone	Poultry
Aminoglycosides	Canada	Neomycin	Cattle
Elfamycine	USA	Efrotomycin	Swine
Glycolpids	Canada	Babermycin	Breeder, turkey
	USA	Babermycin	Swine , poultry
Ionophores/Polyethers	Australia	Lasalocid, Monensin, Narasin Salinomycin	Cattle Cattle Pigs, cattle
	Canada	Lasolocid sodium Monensin Narasin Salinomycin sodium	Cattle Cattle Swine Swine, cattle
	European Union	Monensin Salinomycin	Cattle Pigs
	USA	Monensin, Lasalocid	Cattle
Lincosamides	Canada	Lincomycin hydrochloride	Breeder
Macrolides	Australia	Kitasamycin Oleandomycin Tylosin	Pigs Cattle Pigs
	Canada	Erythromycin Tylosin	Breeder, broiler Sheep

Table 1.9 (cont'd)

	USA	Erythromycin Oleandomycin Tylosin Tiamulin Lincomycin	Cattle Chicken, turkey Cattle, swine, chicken Swine Swine
Oligosaccharides	EU	Avilamycin	Pigs, chickens, turkeys
Penicillins	Canada	Penicillin G potassium Penicillin G procaine	Chicken, turkey Chicken, turkey, sheep
	USA	Penicillin Arsanilic acid	Poultry Poultry
Polypeptides	Australia	Bacitracin	Meat, poultry
	Canada	Bacitracin	Chicken, swine, turkey, chicken
Quinoxalines	Australia	Olaquindox	Pigs
	Canada	Carbadox	Swine
	USA	Carbadox	Swine
Streptogramins	Australia	Virginiamycin	Pigs, poultry
Sulfonamides	Canada	Sulfamethazine	Swine, cattle
	USA	Sulfamethazine Sulfathiazole	Cattle, swine Swine
Tetracyclines	Canada	Chlortetracycline Oxytetracycline	Chicken Turkey, swine, cattle, sheep
	USA	Tetracycline Chlortetracycline Oxytetracycline	Swine Cattle, swine, poultry Cattle, swine

ii. Agencies Involved in AMR monitoring

Some countries have national agencies charged with monitoring antimicrobial usage and rates of AMR in food animals, food and/or people. Examples of such national agencies include:

- National Antimicrobial Resistance Monitoring System (NARMS) in the USA: http://www.cdc.gov/narms/

- Canadian Integrated Program for Antimicrobial Resistance (CIPARS) in Canada: http://www.phac-aspc.gc.ca/cipars-picra/index-eng.php

- Observatoire National de Epidémiologie de la Résistance Bactérienne aux Antibiotiques (ONERBA) in France: http://www.onerba.org/

- The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) in Denmark: http://www.danmap.org/

- Japanese Veterinary Antimicrobial Resistance Monitoring System in Japan

There are also international collaborations that monitor AMR of specific pathogens, such as the WHO Global Salm-Surv, an international program for *Salmonella* surveillance, serotyping and AMR testing throughout the world.

iii. WHO Recommendations for Mitigating AMR in Animals

The World Health Organization (WHO), developed the *WHO Global Strategy for Containment of Antimicrobial Resistance*(116). Key recommendations to address the need for mitigating AMR were listed as follows:

Key recommendations emanating from the 25 expert reports:

- Increase awareness of the antibiotic resistance problem

- Improve surveillance of antibiotic resistance
- Improve antibiotic use in people
- Regulate antibiotic use in animals
- Encourage new product development
- Increase resources to curb antibiotic resistance in the developing world
- Increase funding for surveillance, research and education

The significance of the emergence and continued spread of AMR is sometimes met with skepticism by stakeholders. Some argue that there is not sufficient evidence to prove that AMR may some day bring animal and human medicine back to pre-antibiotic days, and that restrictive regulations on antimicrobial usage are therefore unnecessarily harmful to the animal industries. What is indisputable, however, is that excessive antibiotic usage is known to exert selective pressure on some bacterial populations, that gene swapping among bacteria does occur, and an expanding number of people and food shipments transverse the globe much more quickly than ever before. In addition, development and approval of newer antibiotics has reached a plateau and novel antibiotics are rarely being introduced in the market today. These factors put us all at risk for increasing global AMR problems in future years. Evidence of the trend toward increasing rates of AMR is clear from reports in the literature regarding many previously susceptible pathogens. Taking action at this critical point in our history is important to avoid wasting the efficacy of antibiotics for frivolous purposes whenever good disease control alternatives exist. Veterinarians must do their part to preserve antibiotic efficacy for future generations.

CHAPTER 2

Isolation and molecular characterization of vancomycin-resistant *Enterococcus faecium* from swine in Michigan, USA

Abstract

In 2008 we identified vancomycin-resistant enterococci (VRE) in Michigan swine, which was the first report of VRE in livestock from North America. Continued sampling in 2009 and 2010 was conducted to determine if VRE persisted in Michigan. In 2009, swine manure and feed samples (n=56), county fair pig barn manure samples (n=9), and pooled Michigan State Fair pig barn manure samples (n=18) were screened for VRE. In 2010, swine manure samples were collected from 26 county fairs (n=73) and 9 commercial swine farms in six states (n=28). Recovered VRE isolates were molecularly evaluated by polymerase chain reaction, restriction fragment length polymorphism, pulsed-field gel electrophoresis (PFGE), S1 nuclease digestion, and multilocus sequence typing (MLST). Six VRE isolates were identified in 2009 from the State Fair and another six (8.2%) were recovered from the five county fairs in 2010. All 12 isolates were highly-related to the first reported VRE from Michigan swine: all were confirmed to be vancomycin resistant *Enterococcus faecium* (VREf) carrying *vanA* gene on Tn1546 (Type D), were negative for IS*1251*, *hyl* and *esp* gene, carried a 150-160 kb megaplasmid, and have closely similar PFGE patterns with >80% similarity. Classified as ST5, 6 or 185 by MLST, all belong to the clonal complex 5, a strain recognized to be circulating among European pigs. This

study reveals that VREf are widespread in Michigan swine and persist in the historical absence of the use of agricultural glycopeptides.

2.1 Introduction:

Enterococci are notorious hospital-acquired pathogens. They have natural resistance to a broad array of antibiotics, and are also known for their capacity to acquire mobile genetic elements for additional virulence and resistance. Of particular interest is their acquisition of transferable resistance to vancomycin, a glycopeptide of extreme clinical importance in hospital settings. Based on comparison with a matched hospital population, VRE in patients is associated with adverse outcomes such as increased mortality, morbidity and medical costs (17, 31). The annual number of hospital VRE infections continues to grow, and was estimated to reach 85,586 cases per year in U.S. hospitals (90).

In Europe and elsewhere, VRE has is reportedly widespread in poultry and swine. This was attributed to the extensive use of avoparcin growth promotant , a glycopeptide antibiotic that structurally resembles vancomycin, which was used widely in pigs and poultry prior to its EU ban in 1997. As is the case with humans, colonized animals usually present no clinical signs, can carry the organism for prolonged periods, and can transmit to other susceptible animals and humans. In the U.S., neither avoparcin nor any other glycopeptide was ever approved for use in any food animals. Until our 2008 report, VRE had never been reported in any Western Hemisphere food animals in spite of the widespread prevalence of VRE in hospital settings (7). These first isolates came from Michigan pigs which were raised by 4H club members. The 4H

club is a popular youth organization in the U.S. which is administered by the United States Department of Agriculture and has traditionally emphasized experiential agricultural learning. 4H club members in the livestock program raise animals for the purpose of exhibiting them at county and state fairs.

Our first study objective was to determine if the VRE reported in 2008 was a sporadic finding due to a short-duration, localized colonization of a relatively few swine herds. This objective is key to determining if further research is needed to determine if VRE in US livestock could potentially impact public health. Our approach was to estimate the prevalence of VRE in publically exhibited pigs in Michigan and a convenience sample of commercial swine farms from selected states.

2.2 Materials and Methods:

A. Sample collection, transport and storage

In 2009, swine feeds (n=57) of 4H members, pooled swine fecal droppings from pig barn aisles in five Michigan county fairs (n=9), and pooled swine fecal droppings from the 2009 Michigan State Fair (n=18). For the 2010 study, multi-site manure collection (n=73) coming from pigs exhibited at 26 county fairs were sampled. Commercial herds were sampled by floor manure collection at multiple locations from 2-5 barns at each of 9 commercial swine facilities in Indiana (n=2), Kansas (n=1) Michigan (n=3), Ohio (n=1), Illinois (n=1) and North Carolina (n=1).

All fecal samples were placed in Cary-Blair transport medium (BD Diagnostics Systems, Sparks, MD), following the procedures recommended by the manufacturer. All samples were transported to Michigan State University in ice pack, aliquoted to 2ml cryovials (Fisher Scientific, Denver CO, USA), and then stored at -80°C until transport to the Infectious Disease Research Laboratory at Henry Ford Hospital in Detroit, MI.

B. Isolation, identification, and antimicrobial testing of enterococci

All samples were initially enriched overnight in 5 ml brain-heart Infusion broth and then plated onto Enterococcosel agar (Becton Dickinson, Cockeysville, MD) containing 16 µg/ml of vancomycin and incubated for 48 h at 37°C. Distinct morphological colony types showing blackening due to esculin hydrolysis were subcultured on Trypticase soy agar II (TSAII) (Becton Dickinson, Cockeysville, MD), and confirmed as enterococci using standard biochemical reactions. Antimicrobial susceptibility testing for vancomycin, ampicillin, ciprofloxacin, gentamicin, linezolid, erythromycin, tetracycline, and quinupristin-dalfopristin were determined by E strip (bioMerieux, Solna, Sweden) using CLSI guidelines.

C. Molecular characterization of recovered VRE isolates

All molecular characterization for these isolates followed the same protocols performed in the first VRE report by Donabedian *et al.* (28), which are briefly as follows:

Detection of glycopeptide resistance genes and virulence genes esp and hyl. To determine the vancomycin resistance genotype of the recovered isolates, PCR was performed using the same primers as previously reported for *vanA* and *vanB* (19). PCR was also performed to detect the presence of virulence genes *esp* and *hyl* as described by Vankerckhoven and co-workers (108).

i. Characterization of the transposon Tn1546.

To detect the presence of IS*1251* and determine whether IS*1216V* was combined with the IS3-like element in the left of Tn*1546*, a set of previously reported primers were used (61). To determine whether a previously described base pair variant at position 8234 in *vanX* gene (60) is present in the isolates a procedure previously described by Jensen and others (61) was also performed. Initially, the internal fragment of the *vanX* gene was amplified and the resulting 424-bp product was then digested using DdeI (New England BioLabs, Beverly, MA).

ii. Pulsed-field gel electrophoresis (PFGE)

To determine isolate similarities, genomic DNA of recovered VRE were prepared in agarose plugs and then digested with SmaI (New England Biolabs, Beverly, MA). These were then run on a CHEF-DR III (Bio-Rad Laboratories, Hercules, CA) as previously described (27). To determine percent similarity, Dice coefficient was calculated using the BioNumerics software version 3.5 (Applied Maths, Kortrijk, Belgium) for the banding patterns produced. Isolates were considered related if their PFGE banding patterns were ≥80% similar.

iii. S1-nuclease disgestion

To detect megaplasmids (≥ 150 kb) in VREF from swine PFGE of S1-nuclease- digested genomic DNA was performed following the methods described Freitas *et al* (46), who also performed the same procedure and reported results for the first six VREF isolates (44).

iv. Multilocus sequence typing (MLST)

To determine the evolutionary relationship between isolates, fragments of seven housekeeping genes of *Enterococcus faecium* (*adk*, *atpA*, *ddl*, *gyd*, *gdh*, *purK*, and *pstS*) were amplified following methods as previously described (58). Products were then purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA) and sequenced using the BigDye Terminator version 1.1 cycle sequencing kit (Applied Biosystems, Warrington, United Kingdom). Sequences were then analyzed on an ABI 3100 sequencer (PE Applied Biosystems), and the online eBURST V3 program http://efaecium.mlst.net was utilized to assign a sequence type (ST) to each isolate according to its allelic profile (37).

2.3 Results

For the 2009 study, pooled swine fecal droppings from pig barn aisles in five Michigan county fairs (n=9), and pooled swine fecal droppings in pig pens at the Michigan State Fair (n=18) were examined. All pig feeds and pig barn aisles in selected Michigan county fairs were found negative for VRE (Table 2.1). However, VRE was recovered from a total of 6 of the 18 (33.3%)

pooled fecal samples from the Michigan State Fair pig pens. These pens sequentially housed market hogs and then breeding stocks, where 5/9 (55.6%) and 1/9 (11.1%) of the pooled samples obtained were VRE-positive, respectively (Table 2.2).

Table 2.3 shows the molecular characteristics of the six isolates in 2009. All isolates were confirmed to be vancomycin resistant *Enterococcus faecium* (VREF) carrying the *vanA* gene, and were similar to the previously reported isolates by Tn*1546* characteristics (possess a G-to-T mutation at position 8234 in the *vanX* gene, IS*1216V* combined with the IS*3*-like element at the left end of Tn*1546*, and negative for IS*1251*). The isolates were identified as ST5, 6 or 185 by MLST, all of which belong to clonal complex (CC) 5.

For the 2010 specimens, pooled pig fecal droppings from pigs being exhibited were collected from county fairs at 26 of Michigan's 83 counties (n=73 pooled samples; Figure 2.1). Of these 26 county fairs examined, five (19.2%) were found positive for VRE: Midland, Oakland, Saginaw, St. Clair and Washtenaw (Table 2.4). Together, 8.2% (6/73) of the pooled specimens yielded VRE. The molecular characteristics of the six isolates recovered from this study were similar to the previously recovered VRE from Michigan, as shown in Table 2.5. No VRE were recovered from the 28 pooled manure samples from the 9 commercial farms.

All twelve isolates from 2009 and 2010 were shown to carry an approximately 150-160 kb megaplasmid (Fig 2.2). Analysis of the PFGE banding patterns of these isolates also revealed that all VREf recovered so far, including those from the first report (28), share >80% similarity (Fig 2.3).

All commercial herds sampled from Indiana, Kansas, Michigan, Ohio, Illinois, and North Carolina were negative for VRE (0/28).

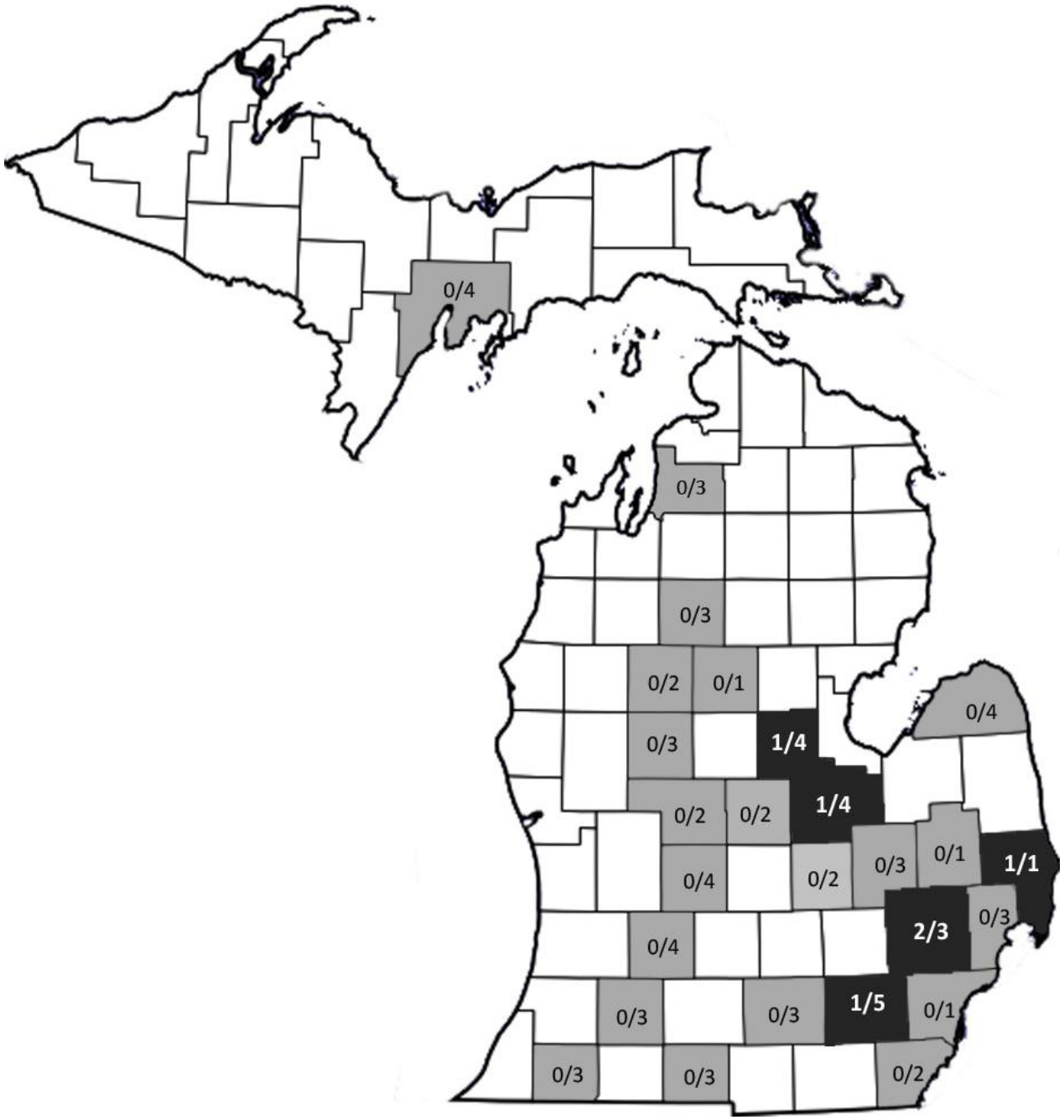


Figure 2.1 Map of Michigan showing the counties tested (n=25) and their respective VRE recovery rates from pooled fecal samples (Legend: Black – sampled counties where VRE was recovered; gray - sampled counties where no VRE was recovered; white – counties where no samples were collected)

Table 2.1 VRE isolation from various samples from different Michigan counties, 2009

Samples	Number of samples collected	Number of samples positive for VRE
Human fecal specimens	56	0
Pig fecal specimens	56	0
Feed fecal samples	57	0
Pig barn aisles, 5 county fairs (pooled)	9	0
Pig pens, Michigan state fair (pooled)	18	6 (33.3%)
Total samples examined	**146**	**6 (4.1%)**

Table 2.2 VRE from State Fair Pig Pens, Michigan, 2009

Type of pigs in pens	Number of pooled samples collected	Number of pooled samples positive for VRE
Market hogs	9	5 (55.6%)
Breeding stocks	9	1 (11.1%)
Total	**18**	**6 (33.3%)**

Table 2.3 Characterization of VRE Isolates from State Fair Pig Pens, 2009

Sample No.	Animal source	Species	*van* gene	MIC: Vancomycin	*IS*1251	IS1216V+ IS-3 like element (left end)	*hyl*	*esp*	MLST	Clonal Complex (CC)
SF1 – 2	Market hogs	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST5	CC5
SF1 – 3	Market hogs	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST185	CC5
SF1 – 6	Market hogs	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST185	CC5
SF1 – 7	Market hogs	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST6	CC5
SF1 – 8	Market hogs	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST6	CC5
SF2 – 4	Breeding stock	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST185	CC5

Table 2.4 VRE isolation from County Fair Pig Pens, Michigan, 2010

County	Number of pooled samples collected	Number of pooled samples positive for VRE
Antrim	3	0
Armada (Macomb)	3	0
Barry	4	0
Branch	3	0
Cass	3	0
Clare	1	0
Delta	4	0
Genesee	3	0
Gratiot	2	0
Huron	4	0
Ionia	4	0
Jackson	3	0
Kalamazoo	3	0
Lapeer	1	0
Marion (Osceola)	2	0
Mecosta	3	0
Midland	4	1 (25.0%)
Missaukee	3	0
Monroe	2	0
Montcalm	2	0
Oakland	3	2 (66.7%)
Saginaw	4	1 (25.0%)
Shiawasee	2	0
St. Clair	1	1 (100 %)
Washtenaw	5	1 (20.0%)
Wayne	1	0
Total	**73**	**6 (8.2%)**

Table 2.5 Characterization of VRE Isolates from various counties, 2010

Sample ID	**County source**	**Species**	***van* gene**	**MIC: Vancomycin**	***IS*1251**	**IS1216V+ IS-3 like element (left end)**	***hyl***	***esp***	**MLST**	**Clonal Complex (CC)**
CF11	Oakland	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST6	CC5
CF12	Oakland	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST6	CC5
CF26	Washtenaw	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST5	CC5
CF29	Saginaw	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST6	CC5
CF32	St. Clair	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST5	CC5
CF65	Midland	*E. faecium*	*vanA*	>=256 ul/ml	-	+	-	-	ST6	CC5

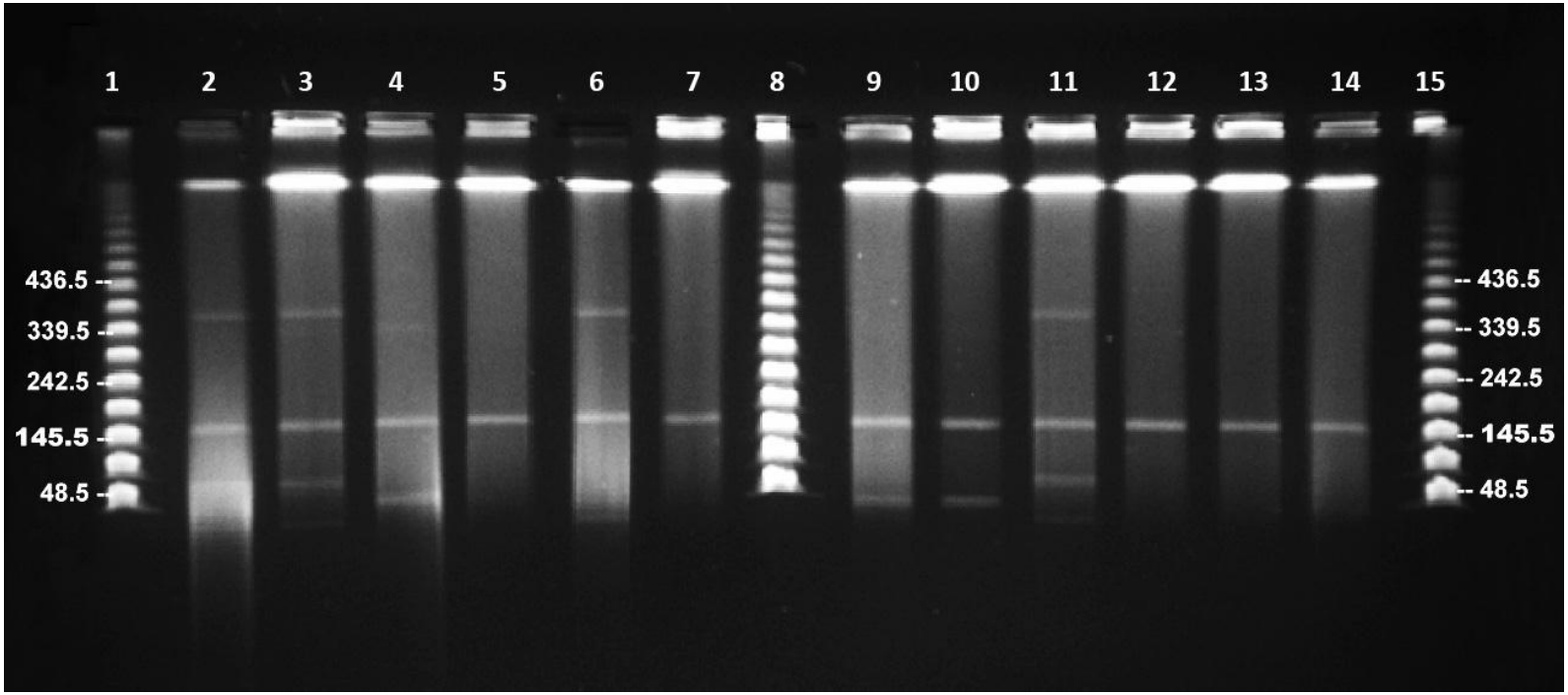
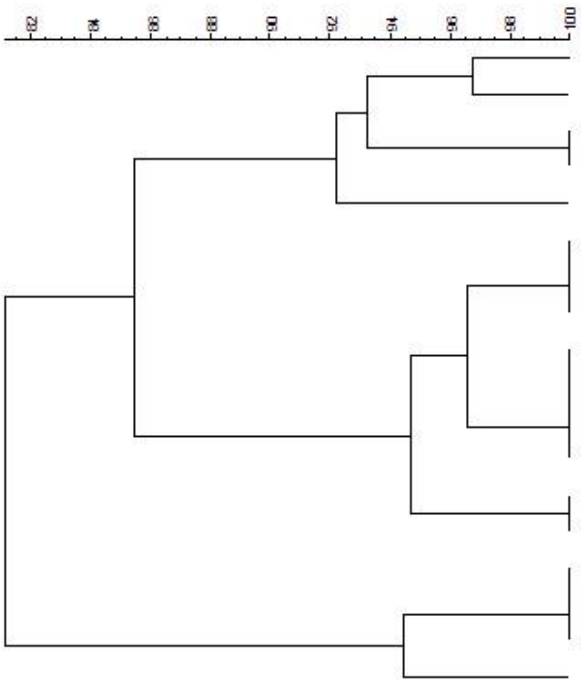
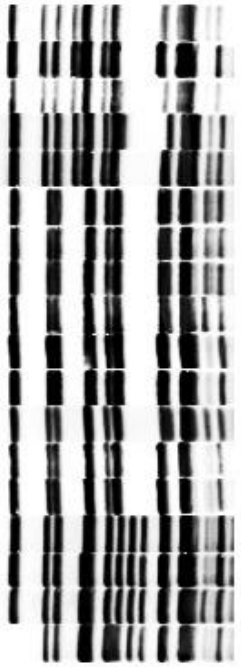


Figure 2.2 PFGE of S1-nuclease digested plasmid DNA showing the presence of a 150-160 kb megaplasmids. Lanes 1, 8 and 15: Lambda ladder marker; Lane 2 – VREf ST5 isolated in 2009 (SF1-2); Lane 3 and 4 – VREf ST5 isolated in 2010 (C32 and C26); Lane 5 to 7 - VREf ST 185 isolated in 2009 (SF1-3, SF2-4, SF1-6); Lanes 9 to 11 – VREf ST6 isolated in 2010 (C11, C12, C29); Lanes 12 to 13 VREf ST6 isolated in 2009 (SF1-7, SF1-8); Lane 14 – VREf ST6 isolated in 2010 (C65). Hybridization studies (image not shown here) for isolates C32, SF2-4, C11, C12, C29 and C65 also further confirmed the *van*A gene is on these megaplasmids.





MLST	CC	Location (Year)
ST5	5	County 3 (2008)
ST5	5	State Fair (2009)
ST5	5	County 2 (2008)
ST5	5	County 6 (2010)
ST5	5	County 5 (2010)
ST6	5	County 4 (2010)
ST6	5	County 4 (2010)
ST6	5	County 4 (2010)
ST6	5	County 7 (2010)
ST6	5	County 1 (2008)
ST6	5	State Fair (2009)
ST6	5	State Fair (2009)
ST6	5	County 8 (2010)
ST6	5	County 1 (2008)
ST185	5	County 1 (2008)
ST185	5	State Fair (2009)
ST185	5	State Fair (2009)
ST185	5	State Fair (2009)

Figure 2.3 Pulsed-field gel electrophoresis dendogram of Sma1 – digested vancomycin-resistant *Enterococcus faecium* from Michigan pigs isolated in 2008 (28), 2009 and 2010. All recovered isolates showed >80% similarity.

2.4 Discussion

This study confirms that the initial first report of VRE in Michigan swine (28) was not an isolated event, but that VRE was found over a 3-year time period in multiple locally-raised pigs herds from multiple Michigan counties. These findings change the initial understanding of the epidemiology of VRE in the United States where it was generally perceived that VRE were only associated with hospital environments (22, 53, 72, 88). As Tables 2.3 and 2.6 show, all 12 recovered isolates are closely highly-related possessing very similar molecular characteristics to the first reported six VRE isolates in the US (28). All 18 VREF isolates recovered thus far from Michigan pigs in the last three years all possess the vanA gene, have a G to T mutation on vanX gene at position 8234, do not have IS1251, possess IS1216V linked to the IS3-like sequence on the left end of Tn*1546*, and have sequence types (ST 5, 6 or 185) that belong to CC5. As with the first six isolates, all possess a 150-160 kb megaplasmids (Fig. 2.2) of which isolates C32, SF2-4, C11, C12, C29 and C65 have all been confirmed to carry the vanA gene through hybridization experiments performed in REQUIMTE, Laborato´rio de Microbiologia, Faculdade de Farma´cia, Universidade do Porto, Porto, Portugal (*Luisa Pexei, personal communication with Susan Donabedian*). Furthermore, all isolates had >80% similarity by PFGE (Fig. 2.3), further indicating that the distribution of VRE among Michigan pigs may largely be attributed to clonal dissemination rather than independent emergence of resistance among enterococcal strains.

The observed molecular characteristics confirm that the Michigan pig VRE isolates closely resemble VRE commonly found in European pigs (44, 45, 58, 61, 86). In fact, the original six isolates by Donabedian *et al.* (28) have been established in a separate study as being similar to

the CC5 epidemic VRE clone which is widespread among swine from different EU countries (44). This molecular and epidemiological evidence suggest that VRE in Michigan pigs may be due to the introduction of VRE from swine or poultry from outside the U.S., their by-products, or other indirect vehicles of transmission (74, 85, 109, 114). Transcontinental spread of VREF is not unexpected, especially since *E. faecium* is recognized for prolonged survival times of many months (74, 85, 109, 114). Live pigs from Europe have been imported into Michigan (89) and VRE have also been previously isolated from animal feeds in the U.S.A. (96). There are no requirements for VRE testing of imported pigs, and visual border inspections for clinical illness would not detect an agent such as VRE which causes no clinical signs in infected pigs. Other means for entry of VRE into the U.S. include international human travel, importation of agricultural products, wildlife, animal pests, or via surface waters (86, 87).

Perhaps a more important question regards why VRE in Michigan swine persists in the absence of any historical or current use of glycopeptides in the U.S. food animal industry. The growth promotant (avoparcin), which was thought to have spurred the emergence of VRE in swine and poultry in other parts of the world, was never used for any agricultural purpose in the U.S. This suggests that the persistence and amplification of VRE in Michigan may be due to co-selection for some other genetic component other than the *van*A gene. Successful survival and dissemination of VRE in a population not exposed to avoparcin has been shown to be possible with co-selection. Studies have shown that genes responsible for mounting resistance against non-glycopeptide antibiotics such as tetracycline, tylosin, and copper, are also located on the same enterococcal mobile genetic element that carries vancomycin resistance (2, 54) in VRE. In the U.S., although prudent antimicrobial use is highly encouraged for livestock, producers

commonly use antibiotics and other additives for growth promotion, prevention and therapy. In the presence of such co-selection, introduced VRE may have been encouraged to persist and flourish in the absence of any glycopeptides usage. The 2008 Michigan isolates have all been shown to carry *tetM* and *ermB* in addition to *vanA* in the same 170 kb-plasmid (44) further indicating that co-selection might have played a role in their survival and persistence.

VRE sequence types belonging to CC5 has been recovered from pigs, piggeries, and slaughterhouses in The Netherlands, Portugal, Denmark, Switzerland and Spain (44, 45, 58). Freitas et al. (44) have also shown that, although adapted to swine, this enterococcal clone is also shared with humans in that isolates have been reported in both hospitalized and healthy humans (44, 58). The VRE strains presently circulating in U.S. hospitals, particularly those related to outbreaks, have reportedly been CC17 (58), although other types such as CC16 (58) have also been identified. VRE types belonging to CC5 have so far not been reported in humans in the U.S., but testing to determine the clonal complex is rarely done. It is therefore conceivable that, especially in Michigan, at least some U.S. human VRE isolates may be due to CC5 strains obtained from pigs.

Although considerably limited in scope, the negative results in commercial farms (0/28), humans in close contact with pigs (0/57), and pig feeds (0/57) may indicate that VRE is not as widespread as it was when it first emerged in Europe.

In addition to these zoonotic CC5 VRE strains further contributing to the already growing VRE problem in U.S. hospitals, another public health concern is the spread of their resistance genes to

other non-enterococal human pathogens. Being located in a mobile genetic element, resistant genes such as van A might be horizontally transferred to other pathogens such as *Clostridium difficile* and methicillin-resistant *Staphylococcus aureus*. The emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA), of which interestingly 80% of the recorded U.S. cases are from South-East Michigan (39, 100), suggests that it may be prudent to prevent propagation of the *van*A gene among all bacterial species. Vancomycin is considered to be the antibiotic of last resort for many drug-resistant hospital-acquired infections, and preservation of its effectiveness is of prime public health importance.

2.5 Summary

In summary, we have recovered VRE from pooled swine fecal samples from Michigan in 2009 and 2010, confirming its continued presence in Michigan swine since it was first identified in 2008. All isolates recovered thus far belong to CC5 and have similar molecular characteristics, suggesting that VRE spread is likely to be due to clonal dissemination. The molecular characteristics of all the Michigan Swine isolates are similar to the swine-adapted enterococcal VRE clone presently circulating in Europe and probably elsewhere. Further investigation is necessary to establish the epidemiology of VRE in U.S. livestock, and to determine how VRE can be best controlled and prevented.

CHAPTER 3

Developing an Open-access Antimicrobial Resistance Learning Site for Veterinary Medical Students

Abstract

Recognizing the crucial role of veterinarians in mitigating antimicrobial resistance (AMR), the Centers for Disease Control and Prevention (CDC) has funded the development of a suite of educational materials to promote responsible veterinary medical use of antimicrobials. An open-access, web-based multimedia curriculum regarding antimicrobial resistance in veterinary practice was thus created. Completed in January 2011, the antimicrobial learning site (AMRLS) for veterinary medical students was made available to the public (http://amrls.cvm.msu.edu/). Designed for integration into existing veterinary medical courses, the AMRLS is also a resource for continuing education of practicing veterinarians, animal scientists and food animal industry specialists. This website emphasizes the mechanisms for the emergence and spread of AMR, the significant role of veterinarians in mitigating AMR, and the need to preserve the efficacy of antibiotics for future generations.

3.1. Introduction

The introduction of antimicrobials in humans quickly led to its widespread therapeutic usage in virtually all companion and food animal species for treatment of various infectious diseases. The

subsequent discovery of Stokstad and Jukes on the growth-promoting activity of antibiotics in pigs and poultry (64, 65, 104) also led to widespread non-therapeutic applications of antibiotics in food animals for growth promotion. Raised in large groups, food animal use of antibiotics have also evolved over the years to include metaphylactic and prophylactic medication.(97) Thus, when the issue of antimicrobial resistance emerged in the years that followed, the extensive use of antimicrobials in animals generated criticism and debates. In particular, the extensive non-therapeutic use of antimicrobials in food animals for growth promotion, metaphylaxis and prophylaxis raised serious public health concern. This highlighted the important public health role and responsibility of veterinarians who are often tasked to assess and advise regarding the conflicting objectives of upholding animal welfare and help sustain agriculture economics, while keeping the interest of human health in mind.

Because there is no existing structured curriculum regarding antimicrobial resistance for health professionals, several campaigns advocating prudent antimicrobial use were instead utilized throughout the world to help improve the judgment of healthcare professionals. Recognizing the need to bridge a similar gap for veterinary medical students and practitioners, CDC has funded the development of a suite of educational materials to promote responsible veterinary use of antimicrobials.

3.2 Materials and Methods

A. Developing the information architecture and conceptual design

The purpose of the website and the target audience was initially defined: this website was created for the purpose of helping veterinary students, the target audience, understand the concept and principles behind antimicrobial resistance. Pre-clinical and clinical veterinary courses in which AMR is taught guided the formation of the AMRLS site map. Each section of the site was treated as an independent module that can be separately integrated into existing veterinary courses where AMR may be discussed.

B. Developing the web content

Web content for the various modules including texts, graphics, and scripts were drafted based on existing literature and recommended good veterinary practices. These drafts were then reviewed and edited internally and externally by experts in the field. Once approved and finalized, the module text files, images, animations, and videos were then uploaded on the AMRLS. Initial layout was done using Microsoft Word and/or Adobe Photoshop. Graphic illustrations were done using Adobe Photoshop and Illustrator. Adobe Flash was used to create animations, interactive media, and quiz questions. jQuery was used to create the image sliders. The AMRLS for veterinary medical students was built using Plone, an open source content management system.

C. Accessibility

The website was designed to be open-access with no requirement for username or password. It was hosted on the World Wide Web under the website of the College of Veterinary Medicine,

Michigan State University. Portable Document Format (PDF) files of modules with static pages were also created for free downloading.

5.3. Results

The website was completed in early 2011, and is now publicly available at http://www.amrls.msu.edu. The modular organization allows for convenient integration into existing veterinary courses and use as homework assignments *in lieu* of traditional reading assignments in text books. Pre-clinical modules cover basic topics regarding antimicrobials, the mechanism of emergence and spread of AMR, and ways in which AMR impacts animal and public health (Table 1).

Table 3.1 Pre-clinical modules in AMRLS

Module	Description
Pharmacology	The pharmacology module presents basic facts on antimicrobials – their historical beginnings, antibiotic classifications, modes of action, applications for animals and prudent use in veterinary practice.
Microbiology	The microbiology module outlines the strategies by which bacteria resist antimicrobials, as well as the molecular basis for resistance. Laboratory methods for measuring antimicrobial resistance are briefly summarized.
Veterinary Public Health	The veterinary public health module describes the impact of the global emergence and spread of antimicrobial resistance in human and animal populations, as well as the impact in soil and aquatic environments. The critical role of veterinary practitioners in mitigating antimicrobial resistance is also emphasized.
Supplementary animated modules	Educational, interactive digital comic books that creatively present the basic concepts of antimicrobial resistance are included as supplementary modules to aid in the learning process.

The clinical modules regard practical veterinary antimicrobial usage and its impact on animal and public health (Table 2).These modules are intended to help current and future veterinary medical practitioners make sound judgments regarding antimicrobial use in animals. Presentations utilize varied learning formats such as animations, audiovisual lectures, case-based presentations, graphic illustrations, interactive quizzes and videos.

Table 3.2 Clinical modules in AMRLS

Module	**Description**
Dairy cattle	Aspects of judicious antimicrobial usage in dairy cattle are presented through various case-based modules: (1) Medicated milk replacer, (2) Neonatal scours, (3) Contagious mastitis, and (4) Model mastitis control program.
Beef cattle	Practical discussions on prudent antimicrobial use in beef cattle are presented in (1) Regression to the mean and (2) Bovine respiratory disease
Pet animal	Case-studies regarding companion animals regard (1) Canine pyoderma, (2) Feline urinary tract infection, (3) Antimicrobial use in companion animal medicine, (4) Rodent multiple-drug resistant *Salmonella* outbreak
Equine	A video case study regards equine respiratory tract infection and the prudent use of antimicrobials.
Swine	An interactive case-study involving *E. coli* infection in piglets is presented

Modules are also downloadable as PDF files for those who prefer to study hard copies or need to review learning materials offline. Each module has a suggestion box for further enhancements and modifications.

5.4 Discussion

Veterinary medical use of antibiotics led to multiple benefits for both animals and humans which include the following: reduction of animal pain and suffering, protection of livelihood and animal resources, assurance of continuous production of foods of animal origin, prevention or minimizing shedding of zoonotic bacteria into the environment and the food chain, and containment of potentially large-scale epidemics that could result in severe loss of animal and human lives. However, it has also become clear that inappropriate antimicrobial use in animals also contributes to the global emergence and spread of antimicrobial resistance. Consequently, this impacts the long-term sustainability of treatment options in both animals and humans. It is thus important that veterinarians possess accurate knowledge and professional convictions regarding AMR and its mitigation. Fanning *et al* (34) even suggested that a modern veterinary medical program should include education regarding antimicrobial resistance.

Because of the widespread availability and acceptance of internet as effective teaching tool (26, 78, 95) and because it has been previously suggested that public health education should combine advances in psychology, communication, and information technology, (33) the AMRLS curriculum was designed to be web-based, open-access, multi-media, and oriented towards the genre of its target audience. With the 4-year veterinary curricula already full (34) and the topic of AMR encompassing multiple courses, the AMRLS was designed for easy integration into existing veterinary medical courses.

Largely through presentation of basic foundational information, case-studies, and examples, the AMRLS helps teach veterinary students to assess and balance competing stakeholder interests

and arrive at treatment plans for selected clinical presentations where antibiotic conservation may be possible. The AMRLS promotes a culture of professional responsibility which emphasizes the veterinarian's critical role in mitigating the emergence and spread of antimicrobial resistance. Although the direct impact of perception on antimicrobial use towards AMR emergence and spread is yet to be measured, it has become evident that perception on antimicrobial usage is influenced by targeted campaigns for appropriate antimicrobial use. For example, Huttner and co-workers identified and evaluated 22 national and six regional campaigns from Europe, North America, Oceania and Israel, observing that most campaigns were effective in reducing antibiotic use (59). Similarly, Bauraind and co-workers reported an inverse association between antibiotic sales and public campaigns in Belgium.(11) Because excessive use of antibiotics is linked to the emergence of resistance, (2, 3, 113) reduction in antimicrobial use, as initiated by campaigns and/or educational tools, may thus indirectly help mitigate AMR development and dissemination.

The benefits of generating AMR awareness in future veterinarians are not limited to the reliance on their voluntarily action to adhere to good veterinary medical practice and reduce the use of antibiotics. Veterinarians educated in the principles, issues and science behind the AMR problem will also be more capable of helping the food animal and companion animal industries understand and support the need for the Food and Drug Administration (FDA) antibiotic regulatory actions to restrict specific antibiotic usages. Enforcement of FDA regulations is often difficult in the face of widespread industry noncompliance, subterfuge or outright refusal.

Some of its modules have been successfully integrated with ease in existing veterinary medical courses in MSU and the U. of Minnesota. Although it varies between modules, it may take an average user about 1-2 hours to complete a module. For further convenience, downloadable PDF files are also available on the site for offline use.

3.5 Summary

The Antimicrobial Resistance Learning site, an open- access, web-based multimedia curriculum regarding antimicrobial resistance in veterinary practice was created to facilitate understanding of future veterinarians regarding antimicrobial resistance. Now publicly available online, campaign for its utilization in more U.S. veterinary medical schools is under way. Although the AMRLS is aimed primarily at U.S. veterinary medical students, this open-access website has potential use for continuing education of practicing veterinarians, animal scientists and food animal industry specialists in the U.S. and elsewhere. To evaluate the website's utility and impact on the target audience, a study regarding the students' knowledge, beliefs and attitudes before and after using the site, is also currently in progress.

CHAPTER 4

Formative, Process and Outcome Evaluation of the Antimicrobial Resistance Learning Site (AMRLS) for Veterinary Students

Abstract

A web-based learning tool for veterinary students regarding antimicrobial resistance (http://amrls.cvm.msu.edu/) was constructed for the purpose of fostering the understanding of the principles, issues and concerns regarding antimicrobial resistance and its mitigation within veterinary medicine. To determine the website's utility, appropriateness, and impact as a learning resource for veterinary students, a preliminary evaluation was conducted about 1 year post-construction. Questionnaires, documentation, and Google Analytics were utilized to determine user feedback, describe marketing and promotional activities, and characterize site traffic for the AMRLS. A student survey was also conducted among veterinary students from Michigan State University (n=90) to determine its efficiency in improving student knowledge, behavioral beliefs, social norms, perceived control, and intentions towards prudent antimicrobial usage in the future. This initial evaluation showed that the AMRLS can be an efficient resource for reaching and teaching veterinary students about antimicrobial resistance and can significantly improve the students' knowledge, attitudes, normative behavior, perceived control, and intentions towards prudence in their future practice. Further improvement on the website's publicity and implementation, as well as addressing technical issues such as site navigation and hardware compatibility may still be needed to further enhance the site and its utility.

4.1 Introduction

The present generation of veterinary practitioners was taught and influenced by veterinarians who practiced veterinary medicine during the period when most currently used antimicrobials were discovered, introduced, and gained recognition as powerful tools in combating diseases. This impressive golden era of antimicrobials, which was immediately followed by the advent of growth-promotion, metaphylactic and prophylactic applications of antibiotics in animal husbandry, fostered a traditional perception of the boundless benefits of antimicrobial application inveterinary practice. This traditional perception may at least partially explain why AMR mitigation has proved challenging as we move into the newage of antimicrobial resistance (AMR).

The Centers for Disease Control and Prevention (CDC) funded the development of a suite of educational materials to promote prudent veterinary antimicrobial usage. The project output was an open-access, web-based multimedia curriculum called the antimicrobial learning site (AMRLS) for veterinary medical students (http://amrls.cvm.msu.edu/). The development process of this site was recently described in the Journal of Veterinary Medical Education (51).

As an essential organizational practice in public health (30), evaluation of this project post-completion was conducted to provide an evidence-based utility of the project's output. Preliminary evaluation of the AMRLS focused on formative, process and outcome evaluation. Formative evaluation included measuring tangible indicators relevant to the site such as: the

number, characteristics, feedback and response of the people using the AMRLS. Process evaluation involved description of marketing/promotional activities related to the project as well as site traffic statistics from Google analytics. The outcome evaluation addressed the effectiveness of AMRLS as a communication tool in fostering prudent antimicrobial use in veterinary practice. To facilitate this, the constructs of the Theory of Planned Behavior (6) was utilized as a rough guideline for assessing whether AMRLS helped modify knowledge, behavioral attitudes, normative behavior, perceived control, and intentions towards AMR mitigation.

4.2. Materials and Methods

A. Formative Evaluation of the AMRLS

Courses from Michigan State University that included selected modules in the AMRLS in their curriculum were identified and described. Numerical and narrative feedback from students based on questionnaires administered via the ANGEL Learning Management System were collected, summarized and/or described.

B. Process Evaluation for the AMRLS

i. Project description

The purpose, underlying theory, objectives, strategies and the expected impact and outcome of the project is described.

ii. AMRLS Marketing, Promotion and Evaluation of AMRLS Development.

Marketing efforts are described to promote the AMRLS via brochure distribution, conference presentations, web advertisement and publication. .

iii. Characterization of site traffic using web analytics.

Using Google analytics (http://www.google.com/analytics), statistics were collected and described regarding the AMRLS website traffic from November 2010 to September 2011. This ten-month period covers the time when the AMRLS was initiated in Google Analytics and the time when this paper was initiated. Data was analyzed regarding site usage, distribution of visits in the United States, distribution of visits around the world, browser capabilities, and traffic sources.

C. Outcome evaluation

i. Subjects for the outcome evaluation.

Veterinary Public Health students for Fall, 2010 at the College of Veterinary Medicine, Michigan State University (n=104) were identified as the pilot class to trial the website.

Fourteen students were excluded in the study for having missed either of the paired (before or after AMRLS viewing) questionnaire, reducing the total sample number to n=90. The study was approved as exempt by the Institutional Regulatory Board (IRB) of the Michigan State University.

ii. Questionnaire design and administration.

The questionnaire was largely based on the constructs of the Theory of Planned Behavior by Ajzen (6) and followed the design described Francis *et al* (43). Prior to this scheduled access assignment, students were given a questionnaire via the ANGEL Learning Management System regarding their knowledge, attitude, social norms, perceived control, and intentions regarding practicing prudent antimicrobial use. Close to the end of the semester, after the students had completed this AMRLS assignment, the same set of questionnaire was re-administered in the same manner with additional questions regarding their feedback on the website. The survey contained ten questions pertaining to their knowledge regarding antimicrobial resistance, five questions each regarding their behavioral attitudes and social norms (with corresponding five valuation/weighting questions for each) five questions regarding perceived control towards prudent antimicrobial use, and nine questions regarding their intentions to practice prudent antimicrobial usage in the future.

iii. Scoring of responses.

Unlike the conventional TPB approach, scores obtained in this study were treated as rank order variables. Responses which were most favorable towards prudent use were given the highest

score of 5, those which were considered as favorable as 4, those which were somewhat in-between favorable and unfavorable were given 3, those which were unfavorable as 2, and responses which are least favorable to prudence were given the lowest score of 1. For attitude and social norm questions, a corresponding motivation rating for each aspect of behavior was also determined. The most favorable answers were given a score of +2, favorable a score of +1, neutral a score of 0, unfavorable a score of -1 and most unfavorable were given a score of -2.

iv. Statistical analysis.

Paired scores (ranks) of the students' knowledge, attitude, social norms, perceived control and intentions were compared pre- and post-exposure to the website by Signed Rank Test using SAS 9.2. For overall category evaluation (knowledge, attitude, social norms, perceived control and intentions), the average difference of the items in the same category were obtained and compared in the same manner.

4.3 Results

A. Formative evaluation

i. ***Characteristics of initial AMRLS audience***

Modules in AMRLS were initially assigned to VM 544 (Veterinary Public Health) which is a required course taught at the College of Veterinary Medicine, Michigan State University. This is

a 2-credit course for second year veterinary students. It covers veterinary environmental, occupational, and public health. Offered Fall semester each year, this course enrolls about 100 students a year. Using a survey questionnaire, the Fall 2010 VM 544 students from CVM, MSU were used as a cluster sample to describe the target audience. The respondents (n= 91) had heard about antimicrobial resistance from Professors (97.80%), the AMRLS for veterinary students (89%), scientific journals (57%), popular media such as news, TV shows and magazines (53%), online sources other than the AMRLS (42%), and conferences (22%).

ii. *Visit distribution by initial users*

Among the available modules, the equine module was the most visited by this class (96.34%) followed by the Ella Salmonella Book 2 (94%), Ella Salmonella Book I (91%), Veterinary Public Health module (90.24%) and the Beef Module (79%). Although not assigned for the course, the students also visited the Dairy Module (20%), the Microbiology module (16%), the Pharmacology Module (5%), the Pet Animal Module (4%), the Integrated Principles Module and the Global Perspectives Module (2%).

iii. Distribution of length of visits of initial users

Most of the students (61%) spent approximately 1 to < 3 hours total with the AMRLS. Others spent < one hour total (9%), 3 to less than 6 hours total (25%), 6 to < 9 hours total (3%), and 9 to < 12 hours total (1%).

iv. Feedback from initial users

Only students who completed this section of the survey were included (n=87). Feedback regarding their experiences, impression and opinions, on AMRLS were obtained through a survey questionnaire (Table 5.1)

Table 4.1 Feedback of Veterinary Students Regarding the AMRLS (n=87)

	Strongly disagree % (95% CI)	**Disagree %** (95% CI)	**Neutral %** (95% CI)	**Agree %** (95% CI)	**Strongly agree %** (95% CI)
I think the web contents are well written.	**1.15** (0.1-5.5)	**3.45** (0.88-9.10)	**18.39** (11.3-27.6)	**62.07** (51.6-71.8)	**14.94** (51.6-71.8)
I think the graphics/layout/visual aids are helpful and appropriate	**2.30** (0.4-7.4)	**4.60** (1.5-10.7)	**14.94** (51.6-71.8)	**58.62** (48.1-68.0)	**19.54** (12.2-28.9)
I think the organization of topics is clear and appropriate	**2.30** (0.4-7.4)	**0.00** (0.0-3.4)	**9.20** (4.4-16.7)	**71.26** (61.1-80.0)	**17.24** (10.4-26.3)
I prefer learning through this manner rather than conventional lectures or from textbooks	**8.05** (3.6-15.3)	**19.54** (12.2-28.9)	**29.89** (21.0-40.1)	**31.03** (22.0-41.3)	**10.34** (5.17-18.1)
AMRLS helped me understand about AMR	**2.30** (0.4-7.4)	**2.30** (0.4-7.4)	**18.39** (11.6-11.3)	**60.92** (50.4-70.7)	**16.09** (9.5-25.0)
AMRLS influenced my perception/ intention/attitude towards AMR	**2.30** (0.4-7.4)	**9.20** (4.4-16.7)	**27.59** (19.0-37.7)	**51.72** (41.3-62.1)	**9.20** (4.4-16.7)
AMRLS helped me appreciate my role as a future vet in controlling AMR	**4.60** (1.5-10.7)	**1.15** (.058-5.54)	**24.14** (16.0-34.0)	**58.62** (48.0-68.6)	**11.49** (6.0-19.5)
AMRLS should be a required reading for vet students	**6.90** (2.8-13.8)	**33.33** (24.0-43.7)	**43.68** (33.6-54.2)	**27.59** (19.0-37.7)	**11.49** (6.0-19.5)
I will recommend the AMRLS to other vet students	**3.45** (0.88-9.10)	**9.20** (4.4-	**49.43** (39.0-	**33.33** (24.0-	**4.60** (1.5-

		16.7)	59.9)	43.7)	10.7)
I will probably re-visit AMRLS in the future for reference or additional learning.	**5.75** (2.1-12.3)	**9.20** (4.4-16.7)	**45.98** (35.7-56.5)	**33.33** (24.0-43.7)	**5.75** (2.1-12.3)

v. Student preferences and dislikes

When asked what they liked most about the site, 25% of the students who volunteered information (n=71) identified Ella Salmonella, for its entertainment value and easy recall. About 23% also indicated that they the manner of presentation of information was really made convenient and easy to understand, as well as the organization and style (7%). Many also (42%) identified the case-based modules as interesting, including beef cattle respiratory module (11%), equine (23%) and specific animal species/and or cases (7%). Students also singled out the following modules presented in the site: Public Health Module (6%), availability of resources (1.4%), the main section (1.4%), dairy module (1.4%), and the illustrations (1.4%), as the most liked for them.

Many students who identified what they disliked the most on the website (n=69) did not like Ella Salmonella comic books (28%) for the reason that these seem to be not suited for their level of education. Others also identified length of modules (23%), technical difficulties such as issues on software compatibility and accessibility (14%), and problems with navigation (7%).

B. Process evaluation

i. Project description

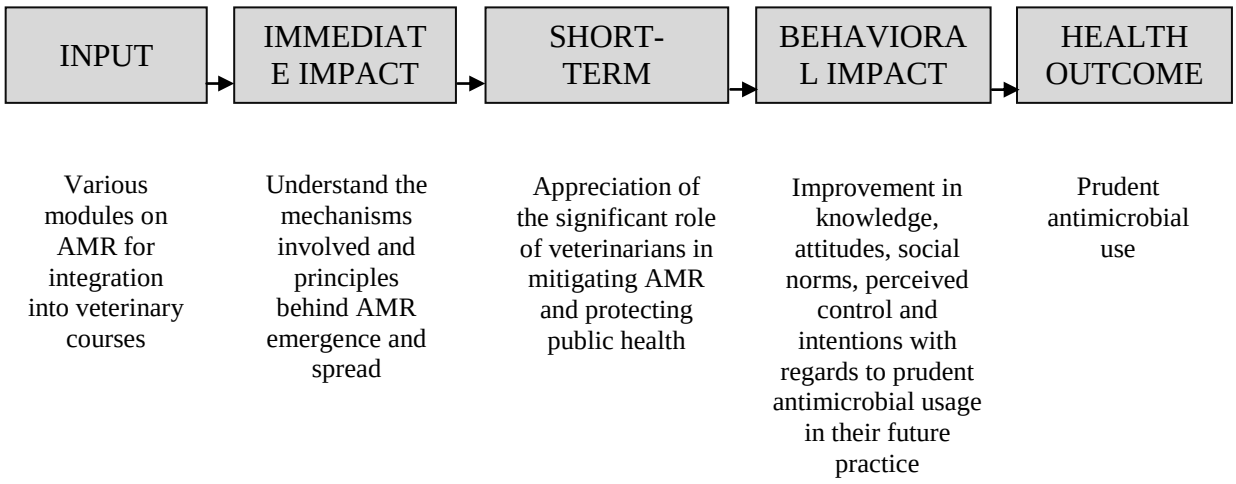
Because of the increasing relevance of AMR in many veterinary courses, the Antimicrobial Resistance Learning Site for veterinary students was designed as a collection of stand-alone modules that can be easily integrated into existing courses. The AMRLS was also designed to be an online, open-access site to make this learning material available to a wider audience. Further details describing the process of development and site features of the AMRLS have been published by Gordoncillo *et al* (51).

The AMRLS was created for the purpose of helping veterinary students better understand the principles and mechanisms behind AMR related to animals and veterinary practice. The short-term impact desired for this project was the appreciation of the significant role of veterinarians in mitigating antimicrobial resistance and their critical contribution in protecting public health. The longer-term objective of the project was that this site will contribute to improving knowledge, attitudes, social norms, perceived control, and intentions of future veterinarians with regards to antimicrobial use in veterinary practice. It is hoped that such positive behavioral change will subsequently translate to prudent use of antimicrobials in veterinary medicine. Following the logic model as described in the example of Saunders *et al* (94), the input, immediate, short-term, and behavioral impacts, as well as health outcomes of the project are as shown in Figure 4.1.

ii. Promotion and Publicity for AMRLS

An integral part of the project is to promote and publicize the site after it has been completed, to engage stakeholders and maximize utility of the output. Since the target audience and those who may be involved in facilitating its use are from the academe, the publicity plan concentrated on venues that will reach this specific population, which were as follows:

Figure 4.1 The AMRLS logic model



1. Brochure: Upon completion, a back-to-back, one-page brochure was printed and distributed to all U.S. veterinary schools to the coordinators of Microbiology, Pharmacology, Veterinary Public Health and Dairy/Beef Cattle Medicine courses This brochure contained the link and abridged information on the background, purpose, contents and other details pertaining to the website. A single-page, soft-copy version of the brochure was also sent as an attachment to veterinary public health mailing lists, and other stakeholders.

2. Scientific conferences. The AMRLS and its modules were also presented in the following scientific conferences in the U.S. and Canada to promote its ongoing development and subsequently, availability:

a. **Symposium on Research in Antimicrobial Resistance in Animal Health and Zoonotic Agents by the Antimicrobial Research Team** at the Center for Public Health, Ontario Canada (September 19, 2008)

b. **Food Safety Symposium** at the 112th Annual Meeting United States Animal Health Association and 51st Annual Conference American Association Of Veterinary Laboratory Diagnosticians in Greensboro, North Carolina (October 26, 2008)

c. **2010 Annual Conference on Antimicrobial Resistance** in Bethesda, Maryland (February 1-3, 2010)

d. **Antimicrobial Stewardship in Canadian Agriculture and Veterinary Medicine Conference** in Toronto, Canada (October 30 - November 2, 2011)

3. Publication. An article entitled "*Developing an Open-access Antimicrobial Resistance Learning Site for Veterinary Medical Students*" which describes the website and its development process was accepted for publication at the Journal of Veterinary Medical

Education. This is now currently in press and is due to be released in the next issue of the said journal.

iv. Web analytics

To describe the extent of reach of the AMRLS, Google analytics was utilized. The site traffic of http://amrls.cvm.msu.edu was evaluated covering a period since the analytics was started (November, 2010) until September, 2011 when this paper was prepared.

1. Site usage. Over the ten-month period, a total of 11,166 visits with 61,088 page views were recorded for the site. On average, visitors view 5.47 pages with page views per visit ranging from 1 to more than 20 pages. Visitors stay on the site for an average of 00:06:01 minutes per visit, and had a 56.25% bounce rate, which is the percentage of single-page visits in which the person left the site from the entrance or landing page. Approximately 35.67% of the recorded visits were from returning visitors and one-time visitors comprise about 64.33% of the total visits.

2. Distribution of visits within the United States. Visitors came from all 51 states over the past year, with Michigan, California, and Minnesota recorded as the three most frequent visitors at 1,426, 1,036 and 944 total visits, respectively (Table 4.2). South Dakota, Delaware, and Michigan had the most pages per visit recorded at 11.44, 11.04, and 10.31, respectively. The states with the longest average time on site per visit were Delaware,

Michigan and Iowa at 22.91, 13.76, and 12.17 minutes. Delaware also holds the record for the least bounce rate at 0.12, followed by Idaho (0.23) and Alaska (0.25).

Table 4.2 Top ten states for AMRLS visits within the US, November 2010 to September 2011

Region	Visits	Pages/Visit	Avg. Time on Site	% New Visits	Bounce Rate
Michigan	1426	10.31	13.76	0.46	0.38
California	1036	2.27	1.69	0.39	0.80
Minnesota	944	8.86	9.22	0.28	0.55
Iowa	447	7.65	12.17	0.44	0.30
New York	297	4.38	4.37	0.75	0.52
Texas	267	4.87	5.92	0.69	0.63
Florida	244	2.29	1.67	0.91	0.77
Illinois	206	5.23	6.25	0.85	0.56
Georgia	176	5.44	5.01	0.69	0.56
District of Columbia	155	5.56	4.35	0.55	0.51

3. Distribution of site visits around the world. The AMRLS has been visited by people from 126 countries thus far, with the United States (7,287 or 65.26%) comprising the largest proportion of the total visits. This is followed by United Kingdom (425 visits or 3.80%), India (373 visits or 3.34%), Canada (331 visits or 2.96%) and Denmark (188 visits or 1.78%). Cayman Islands registered as the country with highest average number of pages visited totaling 18 pages per visit. This was followed by Bahrain (16), Syria (11.83), Ireland (9.89), Ireland (9.89) and Romania (9.29). In terms of longest average time on site, Cayman Islands (23.27 minutes per visit) led the list, followed by Armenia, Somalia, Jordan and Jamaica with an average of 13.04, 12.71, 11.43 and 10.12 minutes per visit, respectively.

Table 4.3. Top ten countries with most AMRLS visits, September, 2010 to September, 2011.

Country/Territory	Visits	Pages/Visit	Avg. Time on Site	% New Visits	Bounce Rate
United States	7287	6.26	7.26	0.56	0.54
United Kingdom	425	3.10	3.44	0.81	0.64
India	373	3.09	2.64	0.88	0.62
Canada	331	6.22	5.35	0.72	0.50
Denmark	199	2.73	2.32	0.75	0.74
Philippines	176	2.96	4.63	0.88	0.63
Australia	174	2.90	2.32	0.80	0.66
Ireland	116	9.89	9.37	0.67	0.30
Malaysia	103	2.49	3.05	0.80	0.56
Brazil	94	8.20	5.43	0.73	0.48

4. Browser capabilities. AMRLS has been viewed via different browsers. The most commonly used was internet explorer (4240 or 38%), Firefox (3361 or 30.10%) and Safari (1977 or 17.70%). Other browsers recorded include: Chrome, Opera, Opera Mini, Android Browser, Mozilla Compatible Agent, IE with Chrome Frame, BlackBerry9300/9000/8520/8530/9650, Camino, Mozilla, HTC_Touch2_T3333 Opera, Netscape, Nokia5235/C1-01/73-1 and SeaMonkey. The most common operating system used for browsing was Windows (8,488 visits or 76.02%), followed by Macintosh (2,385 visits or 21.36%). Other browsers used included: iPhone, Android, iPad, Linux, iPod, Blackberry, Nokia and SymbianOS. Various screen resolutions were utilized for viewing but were mostly 1200x800 (3,695 or 33.09%). The resolution used ranged from 122 x 133 to 3360 x 1050. Viewers mostly had Java support (8,801 or 78.82%).

Most of the service providers utilized for viewing were private companies such as Comcast Cable Communications Inc., Verizon Online Lic, Road Runner Holdco LIc and

many others. Universities also served as service providers for viewing the site the ten most common of which were: Michigan State University (462 visits), Iowa State University (247 visits), University of Minnesota (196 visits), Cornell University (48 visits), University of Missouri-Columbia (41 visits), Western University of Health Sciences (41 visits), Texas A&M University (37 visits), Tufts University (36 visits), Oregon State University (34 visits), and University of Illinois (33 visits). Government and non-government agencies were also recorded as service providers for AMRLS viewing which include: the Danish Network for Research Education (83 visits), the United States Centers for Disease Control (61 visits), Pew Charitable Trusts (45 visits), USDA Office of Operations (42 visits), Public Works and Government Services Canada (37 visits), New York State (28 visits), US Government Accountability Office (15 visits), American Veterinary Medical Association (12 visits), the United States Senate (10 visits) and the World Health Organization (10 visits).

5. Traffic Sources. Sources of visitors were mainly from direct traffic comprising 50.26% of the total site visits. Search engines (mainly including Google, Yahoo, Bing, Search, Ask, and AOL) contributed 35.83% to the traffic generated, while referring sites such as cdc.gov, facebook.com, vetmed.wsu.edu, linkedin.com and many others contributed 13.91%.

C. Outcome Evaluation

To determine whether the AMRLS has the potential for improving the knowledge, attitudes, social norms, perceived control and intentions of veterinary students towards favorable antimicrobial usage, a survey questionnaire was administered before and after the assignment to go through selected AMRLS sites. Results from the survey were summarized and analyzed using SAS 9.2, and are shown in the Tables 4.4 to 4.8 below:

Table 4.4 Difference in student knowledge regarding antimicrobial resistance, before and after AMRLS assignment in VPH class

Knowledge	P value
Awareness that antimicrobials used for animals are also used in humans.	0.1500
Awareness that antimicrobials used for humans are also used in animals	0.2029
Awareness of non-therapeutic use of antimicrobials in animals	0.7500
Awareness of environment contamination of vet antibiotics after these leave the animals.	<.0001*
Awareness of contamination of aquatic environment of veterinary antibiotics	.0002*
Awareness about transference of resistance between microorganisms.	<.0001*
Awareness of transmission of resistant microorganisms in animals to humans	.1195
Awareness that antimicrobial resistance does not always result to fatal consequences	<.0001*
Awareness that all antimicrobials used for animals do not require veterinary prescriptions	.0586
Awareness of the various adverse impact of imprudent antimicrobial usage in animals to human health	<.0001*
OVERALL DIFFERENCE IN STUDENT KNOWLEDGE REGARDING AMR	.0479*

Table 4.5 Difference in student attitudes regarding antimicrobial resistance, before and after AMRLS assignment in VPH class

Attitude	P	Motivation towards attitude	P
Attitude that prudent antimicrobial use will help animal patient/s	.0369*	Positive impression on something that helps animals.	.5610
Attitude that prudent antimicrobial use will help the public health	.0166*	Positive impression on something that helps the public health.	<.0001*
Attitude that prudent antimicrobial use is good veterinary practice.	.0293*	Positive impression on something that engages good veterinary practice.	0.7905

Table 4.5 (cont'd)

Attitude that prudent antimicrobial use will contribute to control of AMR in general	.9012	Positive impression on something that helps control the growing problem of AMR.	.4736
Attitude that prudent antimicrobial use will take effort, time and resources	.1930	Positive impression on something that needs more energy, time and resources at work.	.4157
OVERALL DIFFERENCE	**.1740**		
OVERALL DIFFERENCE IN STUDENT ATTITUDE WITH MOTIVATION TOWARDS ATTITUDE FACTORED IN			0448

Table 4.6 Difference in the students' normative behavior towards antimicrobial resistance, before and after AMRLS assignment in VPH class

Social norm	**P**	**Motivation to comply**	**P**
Will feel pressure from veterinary peers for future prudent use of antimicrobials	0.6792	Importance of the opinion of veterinary peers	.3314
Will feel pressure from federal agencies (such as FDA, CDC, NARMS)	0.7787	Importance of the opinion of federal agencies	.3179
Will feel pressure from the animal industry	<.0001*	Importance of the opinion of the animal industry	.4853
Will feel pressure from the general public	0.9322	Importance of the opinion of the general public	.0202*
Will feel pressure from family/friends/relatives	0.1164	Importance of the opinion of family/friends/relatives	.0337*
OVERALL DIFFERENCE	**.024***		
OVERALL DIFFERENCE IN THE STUDENTS' NORMATIVE BEHAVIOR WITH RELATED MOTIVATION TO COMPLY FACTORED IN			.0129*

Table 4.7 Difference in the students' perceived control over prudent antimicrobial use, before and after AMRLS assignment in VPH class

Perceived Control	P
That they can use antimicrobials prudently if they want to in the future	.0026*
That it would be easy to use antimicrobials judiciously and prudently in vet practice	.0064*
That to use antimicrobials judiciously and prudently, they will need to take into consideration other aspects such as cost, owner's approval, work involved, etc.	.3555
That their decision to use antimicrobials prudently or not in the future, will be entirely up to them.	.3114
That they will probably meet a lot of opposition in the future if they decide to use antimicrobials judiciously and prudently, but they will do it anyway.	.3976
OVERALL DIFFERENCE IN THE STUDENTS' PERCEIVED CONTROL ON PRUDENT ANTIMICROBIAL USE IN THE FUTURE	.0020*

Table 4.8 Difference in the students' intention to practice prudence in antimicrobial usage in the future, before and after the AMRLS assignment in VPH class

Intentions	P
Intention to follow advisories of federal agencies regarding antimicrobial use	0.1090
Intention to be kept informed on public health issues regarding antimicrobial resistance - especially those involving animals	0.6836
Intention to consciously monitor how, when, what and how much antimicrobials are used in their practice.	0.0587
Intention to educate co-workers and clients about antimicrobial resistance.	0.3744
Intention to plan about handling, using, and disposing of unwanted or expired antimicrobials.	0.0315*
Intention to prioritize prudent use of antimicrobials over potential economic benefits of indiscriminant use.	0.1145
Intention to make a conscious effort to understand an antimicrobial's mode of action before using it.	0.3598
Intention to support government for polices that will make all animal antimicrobials for use only by veterinary prescription only, as it is done in some countries.	0.1026
Intention to seek alternatives to the use of antibiotic growth promotants.	0.0076*
OVERALL DIFFERENCE IN INTENTIONS FOR PRUDENT ANTIMICROBIAL USE IN THE FUTURE	.0086

4.4 Discussion

In the recommendations of the American Society for Microbiology Task Force on Antimicrobial Resistance (ASM-TFAR), Jones (63) highlighted the emergent need for education related to antimicrobial resistance, including those targeting human and animal health professionals. Fanning *et al* (34) also described that the current, already crowded veterinary curricula lacks emphasis on this recently emerged challenge to future professionals, particularly on the technical and clinical aspects of antimicrobial use, along with its sociological impacts. Understanding this emerging need and present circumstances, the AMRLS was created and designed in a way that veterinary students in the U.S. and elsewhere will be educated regarding antimicrobial resistance without having to modify their existing course curricula.

Designed as modules that can be integrated into presently existing veterinary courses where antimicrobial resistance may be covered, the website was initially trialed prior to its launch by veterinary students from Michigan State University where the site was created.

Evaluation has been defined as systematic investigation of the merit, worth, or significance of an object (105). This is also considered as necessary for fulfilling CDC's operating principles for guiding public health activities which include: using science as a basis for decision-making and public health action; expanding the quest for social equity through public health action; performing effectively as a service agency; making efforts outcome-oriented; and being accountable (18). For this preliminary evaluation study, three components were used: formative evaluation, process evaluation, and outcome evaluation.

Formative evaluation involves collection of feedback of information relevant to program planning and operation for use in developing and improving the program as it is designed and implemented (25). Formative evaluation in educational technology like the AMRLS regards feedback, revision, review and improvement of product design (115).

Because of the well-recognized impact of antimicrobial resistance on veterinary medicine, it is not surprising that most of the students have heard about this particular topic from sources other than the AMRLS. Both scientific (professors, conferences and journals) and main stream sources of information (other popular online sources, news, TV shows and magazines) have reached these students, indicating the broad availability of information on AMR. The accuracy of information on the contents of these materials, particularly those emerging from pop media, should be scientifically reviewed, considering that their potential bias or misleading content may influence the education of future health professionals.

Most (77%) of the students think that the web contents were well written that the graphics, layout, visual aids were helpful and appropriate (78. %), and the organization of topics was clear and appropriate (88.50%). The majority also considered that the AMRLS helped them understand about AMR (77.01%), that the site influenced their perception, intention and attitude towards AMR (60.92%), and that AMRLS helped them appreciate their role as a future veterinarian in controlling antimicrobial resistance (70.11%). Although it appears that the students have a favorable view of the site, many of them remained neutral as to its role for other veterinary students and their own future utility of the site (Table 4.1). This may be due to the

volume of assigned modules to the students given the short period of time, suggesting that the length of time given and number of modules should be taken into careful consideration when assigning these modules to students.

Generally, modules which were case-based, field-type or downright practical in veterinary practice (42%), appealed to veterinary students. Thus, if there will be future modules added to the site, or a similar project for other health professionals were to be initiated, emphasis should be given to such case examples. Although many liked the Ella Salmonella comic books for its entertainment value and easy recall (25%), a number of the students also consider this material as not fit for their level of education (28%). This contrasting opinion indicates the different learning preferences of students; one important insight however, is that these digital comic books may also find good use for the younger genre or those who have no or little scientific background. Others also disliked the length of the materials covered (23%), which suggests that for these modules to be effective, assigned materials should be given in fitting doses as previously mentioned. The technical difficulties such as issues on software compatibility and accessibility (14%) and problems with navigation (7%), should also be addressed to further improve the site. Such issues may also be addressed in part by the availability of downloadable PDF files of the modules, which are now in place in response to this feedback.

Process evaluation is used to document how well a program has been implemented and to adjust communication activities to meet project objectives (106). It is concerned with documenting and analyzing the way a program operates, to assist interpreting program outcomes, and to inform future program planning (25).

For publicity, the project targeted stakeholders who were generally from the academe. Brochures were directly distributed to veterinary schools in the United States. The website development was also presented at scientific conferences in the US and Canada to reach not only the academic community, but also others who are actively engaged in antimicrobial resistance work. A peer-reviewed journal article regarding the development process of the website was also written and has been accepted for publication at the Journal of Veterinary Medical Education (51). This publication is expected to reach the broader veterinary medical community in the academe. The use of social media, such as linkedin.com, facebook.com, and twitter.com should also be considered for publicity since there is evidence that links from these sites can generate traffic and perhaps promote the availability of the site to the general population.

Results from Google analytics of the AMRLS reveal that, in the brief period that the site went live, it has been visited more than 10,000 times in all 51 states in America (Table 4.2), and 125 other countries (Table 4.3) around the world. This suggests the potential reach of this medium as a teaching resource for veterinary schools, and the need for possible future translation of the contents in other languages. Considering that promotional activities were very limited in scope, these results suggest that its popularity and utility can even be further improved with a more fitting campaign.

The site usage statistics showing that the site been visited more than 1,000 times per month in the last ten months and substantial retaining power given the number of returns (35.67%), page views per visit (5.47 page views per visit), and retaining power (6.01 minutes per visit). There is admittedly a lot of room for improvement to further enhance this statistics. For one, Google

analytics also revealed that there was an average of 56.25% bounce rate, which indicates that the site entrance pages were not very relevant or very compelling to the visitors. Additionally, browsers other than Firefox and Internet Explorer where the site had been tried were also used. The site should thus also be made accessible in other browsers, particularly in mobile devices which are now becoming increasingly popular. The technical construction of the site might also be worth revisiting, considering that two of the key issues raised by students in the trial run were the technical difficulties in accessing some of the site features and ease of navigation.

Although initially created for veterinary students in the U.S., the traffic site indicated visits from 125 countries outside of the U.S. This is particularly encouraging, given that there was no plan or effort to promote the site beyond America. This suggests a wider utility of the site than what was initially envisioned, with no additional costs in the funding support. Translating the site should be considered in the future to maximize the learning opportunity that this free material presents.

Research has demonstrated that behaviors that were assumed to be difficult to change can be modified using well-designed interventions (40). To determine whether the AMRLS had an impact on the students' behavior, an outcome evaluation was also done. Outcome evaluation assesses a campaign's effectiveness and determines whether the campaign achieved its objectives (13). While the formative and process evaluation evaluate systems, procedures, communication processes, and other factors that contribute to the efficient operation of a program, outcome evaluation focuses more on end results or what is ultimately accomplished (106).

The ultimate outcome for this project, which is prudence in antimicrobial use in the future veterinary practice of exposed students, is not immediately measurable. Surrogate outcome measures were thus evaluated instead; this included assessing the immediate, short-term and behavioral impact of the AMRLS that preceded the health outcome, as shown in the AMRLS logic model (Fig 4.1). To achieve this, the constructs used in the Theory of Planned Behavior or TPB (6) were used as a rough guideline for directing the information collection. Unlike the TPB, however, non-parametric testing was utilized for analysis since the values obtained were in rank order (eg: Strongly disagree, Disagree, Partly disagree/partly agree, Agree, Strongly agree). Additionally, for this study, the primary interest is not to determine what predicts the target audience's behavior, but to operationalize the impact of the AMRLS to this audience by determining if there were any significant changes in the students' knowledge, attitudes, social norms, perceived control, and intentions, before versus after AMRLS exposure.

Overall, it was shown that the student knowledge regarding antimicrobial resistance improved significantly ($P = .0479$). Their increased awareness of the impact of veterinary antimicrobials in the terrestrial and aquatic environment, as well as transference of resistance between microorganisms, were particularly pronounced ($P < 0.0001$, $P=0.0002$, and $P < 0.0001$, respectively). This indicates that the AMRLS somehow made them aware that veterinary antimicrobials may actually contaminate the environment and continue its antimicrobial activity, and that, resistance genes can be shared by microorganisms further fostering the emergence and spread of antimicrobial resistance. Their awareness of the various adverse impact of imprudent antimicrobial usage in animals to human health was also enhanced ($P <.0001$), based on their improved scores in identifying potential consequences of imprudent antimicrobial use in animals.

One item that that may need reinforcement and re-emphasizing, however, is that AMR do not always result in fatal consequences.

Attitude toward the behavior is one of the three identified determinants of intention; it refers to the degree to which a person has a favorable or unfavorable evaluation or appraisal of the behavior in question (6). Students' attitude towards prudent antimicrobial use being helpful to patients and public health both had significant difference before and after AMRLS viewing (P = .0369 and P = .0166, respectively). The same was observed for the attitude that prudent antimicrobial use is part of good veterinary practice (P = .0293). Overall attitude, calculated as described above for knowledge, was initially not significantly different (P = .1740). However, when motivation towards each particular attitude was factored in by multiplying scores to the degree of importance they place on that subject of interest (eg: attitude that prudent antimicrobial use will help animal patient/s only becomes meaningful if the student have positive impression on something that helps animals), the overall difference in student attitude significantly improved (P = .0448). It is interesting to note too, that among all the subjects of interest, the students had significantly improved on their impression for something that helps the public health, before and after AMRLS viewing (P <.0001), indicating that their appreciation of something that helps the public health somehow improved after AMRLS viewing.

Subjective norm is a function of normative beliefs, which represent perceptions of specific significant others' preferences about whether one should or should not engage in a behavior (21). It appears that students had significant changes in the degree of pressure they felt from the animal industry (P < .0001). After viewing the AMRLS, the importance of the opinion of the

general public and their family, friends and relatives, differed significantly (P = .0202 and P = .0337, respectively). Overall, with and without their motivation to comply factored in, their social norm differed significantly (P = .024 and P= .0129, respectively). This indicates that their tendency to conform to normative beliefs, brought in part by their improved regard to other members of the society around them, was generally enhanced after viewing the site. Norms carried over during early phase of professional development have been shown to have more long-term influence on health practitioners with regards to antibiotic prophylaxis (73); this thus indicate that shaping of the normative beliefs of these future veterinary practitioners at this stage of their professional training, is worth exploring.

Judgments of perceived behavioral control are influenced by beliefs concerning whether one has access to the necessary resources and opportunities to perform the behavior successfully (6). There were significant improvement in the students' perception regarding their freedom to choose to use antimicrobial prudently in the future (P = .0026) and the relative ease this choice will be for veterinary practice (P = .0064). Overall difference in the students' perceived control on prudent antimicrobial use in the future (P=.0020) indicate that they perceive that they have access to the necessary resources and perceive that there are opportunities (or lack of obstacles) in using antimicrobials prudently. This may indicate that most of the students are likely to perceive high degree of behavioral control (6) in the future. Perceived control by health care practitioners is important, as this has been found to influence their intention regarding prudent antimicrobial use (23).

The students' intentions to practice prudence in antimicrobial usage in the future were also measured before and after AMRLS viewing. Individually, although all aspects of intention showed positive improvement, there were only a couple of intentions that has significantly improved – their intention to plan about handling, using and disposing unwanted or expired antimicrobials (P = .0315) and their intention to seek alternatives to the use of antibiotic growth promotants (P = .0086). Overall, their intentions to practice prudently in the future significantly improved (P = .0086). This also coincides with the large proportion (60.92%) of students claiming in the formative evaluation survey that the AMRLS did influence their perception, attitudes and intentions regarding AMR (Table 4.1)

4.5 Summary

Educational interventions are a cornerstone among efforts to control antibiotic resistance (38). The formative, process, and outcome evaluation of AMRLS post-construction provided an insight that this open-access learning site is a promising and accessible educational tool to help veterinary students in the U.S. and other countries understand AMR issues and principles that may facilitate future voluntarily actions to adhere to good veterinary medical practice and reduce the use of antibiotics in the future. More importantly, veterinarians educated in the principles, issues and science behind the AMR problem will also be more capable of helping the food animal and companion animal industries understand, support, and promote the need for the Food and Drug Administration (FDA) antibiotic regulatory actions to restrict specific antibiotic usages. Areas that may need improvement include: site publicity, addressing technical issues identified in this trial run, and possibly translation of the site to other languages.

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