A framework for assessing the efficacy of antimicrobials in the control of necrotic enteritis in broiler chickens

Gloria Chan,∗ Alessia Guthrie,∗ Theva Sivaramalingam,∗ Jeff Wilson,∗ Dieter Vancraeynest,† Robert Moody,† and Steven Clark†,1

∗Novometrix Research Inc., 4564 Nassagaweya-Puslinch Townline, Moffat, ON, Canada L0P 1J0; and †Zoetis Inc., 1040 Swabia Ct., Durham, NC 27703

Primary Audience: Poultry Farm Managers, Veterinarians, Poultry Scientists

SUMMARY

This review presents a framework for assessing the efficacy of antimicrobials used to control necrotic enteritis (NE) caused by Clostridium perfringens (CP) in the context of susceptibility testing and clinical efficacy, and their potential interactions with the intestinal microbiota of poultry. Practitioners have traditionally based their choice of antimicrobial agent on antimicrobial susceptibility testing, but there appears to be a lack of correlation with clinical efficacy for in-feed antimicrobials (particularly bacitracin and virginiamycin). Resistance patterns of CP and antimicrobials have been monitored using epidemiological cutoffs for minimal inhibitory concentration (MIC), which are not intended to guide therapy. Also, most data have been determined using CP isolates from healthy birds (i.e., potentially from commensal strains not known to be clinically relevant). It is believed that NE is caused by specific virulent CP strains (and potentially other bacteria) that proliferate and displace these commensals. The presence of resistant commensals is not necessarily detrimental (and may even be beneficial) if they inhibit the single CP strain dominance effect observed in acute NE. The choice of antimicrobial therapy in a clinical setting should thus be based on a variety of factors, including an accurate diagnosis, results of efficacy studies, prior experience at the premises in question, and interpretation of MIC data, recognizing that it is not necessarily well correlated with clinical efficacy.

Key words: antimicrobial, Clostridium perfringens, necrotic enteritis (NE), broiler, performance

DESCRIPTION OF PROBLEM

Clostridium perfringens (CP) are ubiquitous gram-positive anaerobes found in the environment and the gastrointestinal tract (GIT) of humans and animals. These anaerobic bacteria are divided into 5 toxinotypes from A to E according to differential production of the major enteric toxins alpha, beta, epsilon, and iota. In addition to these major toxins, CP may produce a variety of other potent toxins, including enterotoxin, beta2 toxin, NetB toxin, and possibly other toxins yet to be discovered. Type A CP, which produces the major toxin alpha (and netB, among other toxins), is associated with avian necrotic enteritis (NE), a widespread disease affecting broiler chickens that is estimated to levy over US$2 billion per year in production costs globally [1]. Control strategies for NE typically include minimizing exposure to known...
risk factors (diet, litter quality, and coccidiosis) and using preventative or therapeutic antimicrobials that have demonstrated efficacy against CP [2–4]. For decades, antimicrobials have been used extensively in North American broiler production and elsewhere to prevent NE and improve performance. Concerns that this practice has contributed to increased bacterial resistance to antimicrobials have led to reassessing control strategies for NE. Practitioners have traditionally used in vitro antimicrobial sensitivity testing to decide which antimicrobial therapy to use to prevent or control NE [5]. However, current research has begun to reveal that data related to specific pathogens’ resistance may not provide a complete picture of the situation in terms of evaluating treatment approaches because poultry intestinal health is now thought to be maintained by interactions (i.e., mutualism and pathogenicity) among a largely unknown and diverse microbiota [6, 7]. Recent investigations have shifted to focus on understanding the efficacy of antimicrobials in vivo, including NE infection models and controlled field trials that measure a variety of parameters related to broiler intestinal health and performance. An emerging area of research in poultry intestinal health involves studies that characterize the normal GIT microbiota (including CP) and the ways in which it is altered when antimicrobials are included in the diet [6]. This review presents a framework for assessing the efficacy of antimicrobials used to control NE in the context of CP susceptibility patterns, in vivo efficacy studies, and our current understanding of NE pathogenesis and poultry intestinal microbiota that may be critical in developing effective control measures.

**IN VITRO SUSCEPTIBILITY TESTING**

Clinicians use susceptibility testing results to determine optimal antimicrobial therapy based on the assumption that the antimicrobial must reach a sufficiently high concentration to inhibit growth or kill an organism [5]. Several methods are available for in vitro antimicrobial susceptibility testing, but methods commonly used in research settings include agar dilution, broth microdilution, and Etest, which yield data on minimal inhibitory concentration (MIC). Benning and Mathers reported low agreement between the agar dilution method and broth microdilution method when determining MIC values for bacitracin and tylosin [8]; therefore, the test method may need to be considered when interpreting susceptibility data.

Based on in vitro susceptibility testing results, antibiotics that have been considered for control of CP infection generally include amoxicillin, benzylpenicillin, bacitracin zinc, bacitracin methylene disalicylate (BMD), lincomycin, tetracyclines (chlortetracycline and oxytetracycline), tylosin, and virginiamycin [9]. Ionophore anticoccidials such as narasin and salinomycin have also been recommended [9]. However, it is important to note that there are currently no validated resistance breakpoints for pathogens that cause enteric disease in poultry [5]. Epidemiological cutoffs have been used to monitor CP resistance patterns [5] in studies that evaluated the MIC distributions of large isolate collections [10–17]. According to Rubin, an organism can have a MIC below the epidemiological cutoff for a particular antimicrobial and be clinically resistant or have a MIC above the epidemiological cutoff remain susceptible; these values should not be used to guide therapy [5]. The proportion of CP resistance to key antimicrobials obtained from the results of epidemiological studies is summarized in Table 1. Most antimicrobials used to prevent enteric infection in commercial broiler production are administered in feed [18]. The current review emphasizes a comparison of bacitracin, virginiamycin, and tylosin because they are the most widely used in-feed antimicrobials.

**Bacitracin**

Generally, CP isolates have been classified as resistant to bacitracin for MIC values >16 μg/mL [11, 14]. Llanco and colleagues used a resistance cutoff value >8 μg/mL [19], and other studies determined resistance by visual inspection of MIC graphs [13, 15, 17]. In most studies, CP isolates were obtained from healthy birds [11, 13, 17, 20]. Chalmers and colleagues’ study [14] specified whether CP isolates came from healthy birds or birds affected by NE, although with the exception of one reference strain known
to be virulent, it was not known whether the isolates were specifically responsible for causing the NE outbreak. Llanco and colleagues also attempted to evaluate the antimicrobial susceptibility of CP isolated from healthy birds and birds affected by NE [19]. However, the results may not be representative because CP could be isolated from only a portion (9.4%) of intestinal samples belonging to NE-affected birds, and it was not known whether the recovered strains were virulent [19]. The isolates could not produce NetB [19], a toxin believed to be important in NE pathogenesis [14].

Reported resistance ranged from 0 to 15% in Europe [11,13], 47.3 to 50% in Brazil [15,19], 45.9 to 95% in Canada, and 88.5% in the United States [10,14,17,20] (Table 1). Interestingly, several studies indicated that bacitracin had distinct bimodal distributions of MIC (i.e., a population of CP isolates was susceptible and another population was resistant) [10,15,17], although there were slight differences in MIC values depending on the testing method. MIC values for bacitracin can be at least 2 dilutions higher when a broth microdilution (rather than an agar dilution) is used [8]. The cause for widespread bacitracin resistance (based on MIC values) is unknown, but it was suggested that resistance could be spread horizontally between the strains present in the barn or through the continuous selective pressure of bacitracin in the feed (which could be the function of issues such as production practices, geography, etc.) [20]; Chalmers and colleagues speculated the latter hypothesis to be true; in a separate study, however, Chalmers and colleagues [14] reported that CP isolates obtained from antimicrobial-free birds also demonstrated bimodal patterns of resistance. Therefore, the hypothesis of horizontal spread through specific resistant determinants may also be warranted [20]. Interestingly, Chalmers and colleagues also observed that the reference virulent isolate obtained 10 y previous to their study was identical to an isolate they obtained from a bird affected by NE that was susceptible to bacitracin [14]. This may indicate that the same CP clones can persist for many years [14] and perhaps maintain a similar

<table>
<thead>
<tr>
<th>Country</th>
<th>Reference Test1</th>
<th>AMP3</th>
<th>BMD4</th>
<th>CTC5</th>
<th>LIN6</th>
<th>MON7</th>
<th>NAR8</th>
<th>OTC9</th>
<th>PEN10</th>
<th>TYL11</th>
<th>SAL12</th>
<th>VIR13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>12 AD</td>
<td>66.0</td>
<td>63.0</td>
<td>66.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Belgium</td>
<td>13 AD</td>
<td>47.3</td>
<td>3.6</td>
<td>61.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Brazil</td>
<td>15 AD</td>
<td>50.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Brazil</td>
<td>19 AD</td>
<td>95.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Canada</td>
<td>20 BD</td>
<td>45.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Canada</td>
<td>14 Etest</td>
<td>64.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Canada</td>
<td>17 BD</td>
<td>15.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Norway</td>
<td>11 BD</td>
<td>3.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sweden</td>
<td>11 BD</td>
<td>88.5</td>
<td>57.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>United States</td>
<td>10 BD</td>
<td>95.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

1 AD: Agar dilution method; BD: Broth microdilution
2 For each antimicrobial, the numbers represent as the percentage of isolates classified as resistant (-: indicates no data available)
3 AMP = ampicillin
4 BMD = bacitracin methylene disalicylate
5 CTC = chlortetracycline
6 LIN = lincomycin
7 MON = monensin
8 NAR = narasin
9 OTC = oxytetracycline
10 PEN = benzylpenicillin
11 TYL = tylosin
12 SAL = salinomycin
13 VIR = virginiamycin
susceptibility pattern. The potential significance with respect to interpreting MIC values of CP clonality and their bimodal MIC distribution for bacitracin is discussed in more detail later in this review, as is potential selection of healthy versus diseased birds for MIC determination.

**Virginiamycin**

Johansson and colleagues categorized CP isolates as resistant to virginiamycin for MIC values $>8 \mu g/mL$ in which a bimodal distribution was observed [11], although a lower (visually determined) cutoff was used by Slavic and colleagues when the isolates did not show a bimodal pattern of MIC distribution [17]. A low resistance level for CP was reported in Europe (from 0 to 13%) [11]; the resistance level in Canada was 25% [17] (Table 1). In these studies, CP isolates came from healthy birds. In the United States, Watkins and colleagues determined that CP isolates were susceptible to virginiamycin [10]; however, 31% of the isolates had MIC values of 16 $\mu g/mL$, which, using Johansson and colleagues’ cutoff, would be classified as resistant (Table 1) [11]. In the latter study, some isolates came from flocks with a history of necrotic enteritis, but it was not specified which isolates came from diseased birds [11].

**Tylosin**

Susceptibility studies have generally reported that all CP isolates are sensitive to tylosin [10, 12, 13] (Table 1); therefore, an epidemiological MIC cutoff point for resistance has not been established. For tylosin, MIC values $\geq 1 \mu g/mL$ with an extended frequency distribution range were observed in Europe [12, 13], and MIC values between 2 and 4 $\mu g/mL$ were observed in the United States [10].

**Other Antimicrobials**

The sensitivity of CP isolates has been reported for amoxicillin and benzylpenicillin, as well as anticoccidial ionophores such as salinomycin, narasin, and monensin [10–12, 15, 16] (Table 1). Due to the low level of CP resistance observed among these antimicrobials, studies have not determined resistance cutoff values. Increasing MIC values ($\geq 1 \mu g/mL$) were observed for lincomycin with an extended frequency distribution range, which is suggestive of acquired resistance [10, 12, 13, 16] (Table 1). Martel and colleagues reported that chlortetracycline was active on CP at very low concentrations compared with its relatively low activity to other bacteria; in addition, chlortetracycline was more active than oxytetracycline on CP [12]. A low-level resistance to the tetracyclines was observed in 66% of the CP isolates (Table 1) when resistance was visually defined by isolates belonging to the higher range of MIC ($>0.12 \mu g/mL$ for chlortetracycline and $>1 \mu g/mL$ for oxytetracycline) [12]. Johansson and colleagues categorized CP isolates as resistant to oxytetracycline for MIC values $>2 \mu g/mL$ [11]. In that study, resistance to oxytetracycline was observed in strains isolated from Sweden (76%), Denmark (10%), and Norway (29%) [11] (Table 1).

**EFFICACY STUDIES**

The efficacy of several antimicrobials in the control of NE has also been evaluated through several NE infection models [21–31] and a few controlled field trials [18, 32] that measured a combination of different performance parameters, including mortality, feed conversion ratios (FCR), weight gain, and/or the presence and severity of NE lesions. It is important to note that the correlation between in vitro susceptibility data and the clinical efficacy of antimicrobials has not yet been investigated [17].

**Necrotic Enteritis Infection Models**

Brennan and colleagues investigated the clinical efficacy of BMD in combination with narasin in a CP inoculum challenge model and reported that improvements in NE-associated mortality and lesions were greatest for BMD- and narasin-medicated birds, followed by BMD alone and then narasin alone [24]. It was also reported that BMD and narasin fed alone and in combination provided significant improvements in ADG over the entire study compared with untreated (challenged and nonchallenged) birds; the authors suggested that the BMD’s positive effect was likely related to NE control (i.e., reduction of NE lesions) and not simply a result of a growth...
promoter effect [24]. Using a similar NE model, Shojadoost and colleagues reported that using virginiamycin and salinomycin alone or in combination also prevented NE-associated mortality; birds fed virginiamycin alone maintained a similar BW to nonchallenged birds and had improved weight gain compared to challenged birds [28].

Cravens and colleagues focused on NE, aflatoxicosis, and treatment interactions with virginiamycin on broiler chick performance [27]. Virginiamycin improved feed intake, weight gain, and feed conversion; it decreased mortality [27]. However, even though performance was improved, challenged birds fed virginiamycin also had unexpected higher NE-specific lesion scores than challenged birds not fed virginiamycin; this contradicts results reported by Shojadoost and colleagues [28]. Cravens and colleagues suspected that increased lesion scores were due to intestinal wall thinning and decreased GIT length and weight [27].

The NE model proposed by Collier and colleagues used molecular (16S rRNA gene-based) and culture-based techniques to investigate the effects of tylosin on the community structure of small intestinal microbiota relative to CP colonization [25]. The study reported that tylosin administration quantitatively decreased the proportion of mucolytic bacteria in general and of CP specifically (which is particularly mucolytic); these responses correlated with reduced occurrences of NE lesions and improved intestinal barrier function [25]. Similarly, Brennan and colleagues reported that tylosin was effective in treating clinical outbreaks of NE and improving challenged birds’ performance [23].

Other NE models have demonstrated the clinical efficacy of antimicrobials used in CP therapy, including amoxicillin, benzylpenicillin, chlorotetracycline, lincomycin, bacitracin zinc, and the ionophore anticoccidials lasalocid, narasin, and salinomycin [16, 21, 22, 25, 26, 29–31].

**Controlled Field Trials**

LaVorgna and colleagues conducted a field trial to compare the performance of broilers fed BMD or virginiamycin over 3 consecutive flock cycles [18]. In the first cycle, virginiamycin-treated birds demonstrated improved feed conversion and processing weights compared to BMD-treated birds, but the effect was not maintained in later cycles [18]. The authors reported an unexpected 4% increase in non-NE mortality of virginiamycin-treated birds in cycle 3 compared to BMD-treated birds, which may also have affected the performance results in the virginiamycin-treated groups because the dead birds were replaced in the study to maintain equal stocking densities [18]. Virginiamycin has broader-spectrum activity against gram-positive bacteria compared to BMD, therefore the authors speculated that increased mortality was in part due to suppressed beneficial bacteria that resulted in the emergence of pathogens [18]. Because in their study, the benefits of virginiamycin on performance were not maintained across flock cycles, LaVorgna and colleagues advised that single flock efficacy studies should be interpreted with caution [18]. Miles and colleagues compared GIT morphology changes in birds fed BMD or virginiamycin; birds fed in both groups had increased body weight and decreased intestinal length and weight compared with control birds, but the effect on intestinal length and weight was greater in birds fed virginiamycin [32]. Because bacitracin and virginiamycin have different modes of action and spectrum of activity on gram-positive bacteria [32], it can be speculated that changes in GIT morphology may be modulated in part through unknown interactions with host factors and/or intestinal microbiota. Further field studies may lead to better a understanding of the long-term impact of preventative antimicrobial use on broiler performance in relation to NE control.

**MICROBIAL INTERACTION AND DIVERSITY STUDIES**

Investigations aimed at understanding the complex interactions among antimicrobials, CP subpopulations, and other bacterial species comprising the poultry intestinal microbiota may be critical in developing effective NE control strategies. It is important to note that not all CP strains can induce NE, but the strains must possess (and express) specific virulence factors to induce NE [33]. Insults to the GIT environment may create...
conditions that allow virulent CP proliferation (up to $10^6$ to $10^8$ cfu/g) and produce a spectrum of effects that include subclinical infection with focal intestinal necrosis and liver disease or the classic clinical form of acute fulminant necrotizing enteritis [2, 3].

**Diversity and Interaction of Clostridium Perfringens**

Type A CP is a diverse population comprised of different subtypes that appear to change over time within the poultry GIT [20]. Researchers have investigated the genetic diversity of CP strains through different molecular typing techniques, particularly pulse-field gel electrophoresis (PFGE) and multilocus variable number of tandem repeats analysis (MLVA) [14, 20, 34–36]. For example, Chalmers and colleagues characterized the population diversity of CP in healthy birds and observed how it changed over time on a commercial farm at which bacitracin was fed to birds in one barn and withdrawn from birds in another barn [20]. In total, 8 major PFGE types and 17 subtypes were identified, with no significant effect of bacitracin withdrawal on pulsotype diversity between barns [20]. Chalmers and colleagues also identified a major pulsotype that persisted in the barns for a long time but observed a gradual decrease in its prevalence over time, which could indicate competition among CP strains seeking to populate the poultry GIT [20]. In that study, all CP isolates were resistant to bacitracin except for 2 strains, each of which belonged to unique PFGE types found toward the end of the flock cycle [20]. It can be speculated that bacitracin resistance is maintained among commensal strains of CP.

Other studies have demonstrated that CP diversity is actually greater among healthy flocks compared to NE-affected flocks in which CP populations were often clonal (i.e., they showed identical PFGE profiles) [34–36]. Barbara and colleagues reported that several CP subtypes were found in CP-associated organ lesions in birds without clinical symptoms (i.e., in birds with subclinical infections) [39]. It is tempting to speculate that NE may be partially suppressed via competition among diverse CP subpopulations, including virulent and nonvirulent strains. This theory may challenge current guidelines related to choosing the correct antimicrobial therapy for preventing and controlling NE in which the antimicrobial must sufficiently kill or suppress all CP growth. Given that routine published MIC data are often determined using CP isolates obtained from healthy birds (i.e., those not known to be clinically relevant), it is difficult to interpret the clinical relevance of the MIC values.

**Diversity and Interaction of Other Microbial Species**

The mechanisms through which antimicrobials improve overall intestinal health and performance and also reduce intestinal lesions remain to be elucidated. It is believed that antimicrobials generally kill or impair the growth of bacteria; thus, the number of growth-depressing metabolites and the microbial load that would otherwise compete with the host for available nutrients and cause immunological stress in the intestine are reduced [40]. Antimicrobials also reduce the weight and length of the intestines and result in a thinner intestinal epithelium, thus enabling the host to better absorb nutrients [40].
However, these mechanisms do not entirely capture the role of antimicrobials among the complex interactions of the poultry intestinal microbiota, where only 10% of bacterial species have been identified [7]. Researchers have begun to question the role of microbial diversity in poultry intestinal health, how the interactions of various microbes may be altered by antimicrobial use, and whether these interactions lead to beneficial or detrimental effects.

Although it is widely believed (perhaps erroneously) that NE is best controlled by suppressing all CP growth, it can be speculated that overall general maintenance of healthy poultry intestinal systems is modulated not only by CP alone but also by competition among several bacterial species. Collier and colleagues hypothesized that changes in the GIT (e.g., coccidiosis, highly viscous dietary feedstuffs) result in increased mucus production, which can select for mucolytic bacteria, including CP, and serve as an initiating step for NE pathogenesis [25]. In addition, an inverse relationship was observed between CP and *Lactobacillus gasseri*, which may indicate competitive interaction between these bacteria because *L. gasseri* has been shown to compete for limiting nutrients and exclude pathogenic bacteria via the production of bacteriocins [25]. Gong and colleagues investigated the effects of bacitracin zinc on bacterial microbiota in the ileum and ceca of broilers and reported that bacitracin treatment did not alter microbiotal richness (i.e., bacterial numbers and diversity) [41]; this contradicts the belief that antimicrobials exert an effect in part through reducing microbial load [40]. Microbiotal competition was, however, altered, although the bacterial species corresponding to the composition changes were not determined [41]. Engberg and colleagues reported that treating broilers with bacitracin zinc and salinomycin, alone or in combination, resulted in lower counts of CP and *Lactobacillus salivarius*, and they suggested that high numbers of these lactobacilli may play a role in growth depression due to competition in nutrient uptake or impaired fat absorption caused by bile acid deconjugation [21]. Similarly, Dumonceaux and colleagues showed that virginiamycin increased the abundance of certain species in poultry GIT without significantly altering the total bacterial numbers [6]. These researchers further showed a short-term improvement in feed efficiency and weight gain (over 15 d); thereafter, virginiamycin had no significant effect on performance [6]. These findings place in further doubt the direct value of determining MIC values for CP via selection of antimicrobials for NE control; and these findings may also explain in part the apparently weak correlation between MIC data and the clinical efficacy of antimicrobials in NE infection models and field studies. The composition of bacterial species modulated by antimicrobials should be examined more closely to aid in an understanding of their effects on intestinal health [6].

**DISCUSSION**

The current review provides a framework for assessing the efficacy of antimicrobials in the context of CP susceptibility, clinical efficacy, and potential role in modulating poultry intestinal ecology (an emerging area of research in enteric poultry diseases).

Antimicrobial therapy is currently guided by susceptibility testing based on the assumption that NE control is best achieved by killing or suppressing the growth of CP in general. When assessing results, practitioners may also need to consider the laboratory test method that was used to obtain MIC values (i.e., agar dilution vs. broth microdilution), whether the CP isolates came from healthy or ill birds, and whether there are validated resistance breakpoints for poultry CP infection. The current susceptibility data for CP have been obtained from epidemiological studies [10–17], which are not intended to guide therapy [5]. These studies’ established cutoff values have described antimicrobial susceptibility from the perspective of the organism as opposed to from a clinically predictive perspective: Organisms with MIC values above the epidemiological cutoff have resistant mechanisms that make them less susceptible to an antimicrobial than to other organisms of the same species; this does not necessarily mean they are clinically resistant to the particular antimicrobial [5]. Nevertheless, epidemiological studies provide invaluable information on the changes to CP antimicrobial resistance.
Susceptibility test results have generally demonstrated CP sensitivity to amoxicillin, benzylpenicillin, tylosin, and ionophore anticoccidials (e.g., salinomycin and narasin) [9, 16]. Varying levels of CP resistance have been observed for lincomycin [10, 12, 13, 16]. Low-level resistance has been observed for chlortetracycline, though few sensitivity data are available for this particular tetracycline [12]; low to high levels of resistance have been observed for oxytetracycline [11, 12]. Additionally, many of these therapeutic antimicrobials and ionophore anticoccidials have demonstrated clinical efficacy in controlling NE via infection models [16, 21, 22, 25, 26]. In alignment with the current assumptions of effective antimicrobial therapy, antimicrobials with demonstrated high CP sensitivity appear to control clinical outbreaks of NE by suppressing all CP proliferation, including commensal strains (i.e., most susceptibility studies obtained CP isolates from healthy birds). For example, Collier and colleagues reported that the microbial populations of tylosin-treated birds were more homogenous than and dissimilar to those of birds not treated with tylosin [25].

A lack of correlation becomes apparent when comparing the susceptibility of CP to preventative antimicrobials with their clinical efficacy. Low to moderate levels of resistance have been observed for virginiamycin, whereas high levels of resistance with distinct bimodal MIC distributions have been observed for bacitracin [10, 11, 13, 14, 17, 20]. However, both antimicrobials have demonstrated clinical efficacy in reducing NE-specific mortality and/or improving bird performance via infection models and controlled field trials [18, 24, 27, 28, 32]. For this reason, we suggest that understanding the NE disease process and the role of antimicrobials in modulating intestinal ecology may also help uncover the underlying mechanisms of these antimicrobials and thus lead to a better assessment of their efficacy.

There is evidence that NE is caused by specific virulent CP strains that have competitive advantage over other virulent and nonvirulent CP strains (both of which may be commensal to the poultry intestinal microbiota). Potential factors that confer competitive advantage to CP strains may include the ability to adhere to the intestinal epithelium and/or extracellular matrix molecules [38], and the ability to produce bacteriocins [37], NetB toxin [14], and other virulence factors yet to be discovered. It is possible that controlling NE is partly modulated by competition among diverse CP subpopulations that may suppress the single CP strain dominance effect observed in acute NE [37]. The role of other bacterial species in NE pathogenesis may also be important, for example, certain species of lactobacilli (L. gasseri) may also produce bacteriocins that prevent pathogen emergence [25], while other species (L. salivarius) may exacerbate growth depression [21]. These findings appear to challenge the belief that preventative antimicrobials control enteric infection through a general reduction of microbial load [40], or at least it does not seem to provide a complete picture of the situation of how antimicrobials prevent infection [7]. Furthermore, bacitracin and virginiamycin have been reported to alter the composition of microbial species in the GIT without reducing the complexity of or total bacterial species [6, 41]. Some researchers now believe that any major shifts in poultry intestinal microbiota may allow the emergence of pathogens [18] and that long-term intestinal health may be effectively maintained through a balance of the mutualism and pathogenicity [6] of various bacterial species, including CP subpopulations. The importance of antimicrobial-resistant CP strains is unclear in this context. For example, it is likely that many studied resistant CP strains are commensal organisms that have persisted in the barn environment for a long period and are quick to populate the poultry GIT in the early stages of the flock cycle [20]. It is possible that the presence of resistant commensal strains (with or without virulence attributes) in the poultry GIT is not necessarily detrimental if they inhibit the single-strain dominance effect observed in acute NE outbreaks. On the other hand, antimicrobial therapy could be ineffective in situations in which a virulent CP strain with competitive advantage over other CP strains acquires resistance. The mechanism for the spread of resistance is unknown; however, CP clones may persist for several years, as has been observed by Chalmers and colleagues—a reference virulent strain obtained 10 years prior to their study was identical to a bacitracin-susceptible strain recovered from
a bird affected by NE [14]. Currently, little information is available on the antimicrobial susceptibility patterns of NE-causing CP strains because there are no methods to identify CP pathotypes, and the isolates specifically responsible for causing clinical outbreaks are difficult to recover postinfection [37]. To better assess and understand the importance of antimicrobial resistance by CP, further characterization studies may be required; subsequently, the differences in antimicrobial resistance levels among CP subpopulations could be compared.

From a clinical perspective, NE control measures are based on proper diagnosis, management of known risk factors, and prudent use of antimicrobials. According to Wilson and colleagues, diagnosing NE is challenging and involves a comprehensive analysis of flock history and condemnation records, clinical signs, gross and histopathology, and bacterial culture [2]. Susceptibility test results should also be interpreted with caution because the isolated organism may not be responsible (or fully responsible) for causing the disease. Another important factor to consider is the empirical response of the flock to previous changes in management (control of coccidiosis, diet, and litter quality) and/or choice of antimicrobial therapy [2]. In an effort to reduce inappropriate and excessive antimicrobial therapy, guidelines for prudent use have been established by Weese: 1) use antimicrobials when there is a bacterial infection, 2) select antibiotics based on an exact diagnosis, 3) limit the spectrum of activity of the antimicrobial as narrowly as possible and have a high margin of safety, and 4) adhere to label instructions (e.g., do not underdose or prolong the dosing interval) [42]. In addition, novel technologies to prevent NE are emerging, for example, competitive exclusion products, probiotics, prebiotics, organic acids, enzymes, plant extracts, hen egg antibodies, bacteriophages, and vaccination [43]. Further research to characterize and/or enumerate bacterial species comprising poultry intestinal microbiota should lead to the development of improved practical diagnostic tools for NE and other poultry enteric diseases with the potential for widespread adoption, and increasingly effective approaches to improvement of intestinal health.

CONCLUSIONS AND APPLICATIONS

1. Antimicrobial susceptibility data (i.e., MIC data) for CP have been obtained from epidemiological studies, which are not intended to guide therapy.

2. Studies evaluating antimicrobial resistance of CP were largely conducted on isolates obtained from healthy birds (i.e., strains not known to be clinically relevant), which may partly explain the lack of correlation between MIC data and antimicrobial (particularly bacitracin and virginiamycin) in vivo efficacy data obtained from NE infection models and field studies.

3. Acute NE appears to be caused by the proliferation of specific virulent CP strains. We suggest that pathogenesis may be suppressed by competition among diverse CP subpopulations and other bacterial species (e.g., lactobacilli). Resistant CP subpopulations that are commensal to the poultry microbiota are not necessarily detrimental (and may even be beneficial) if they inhibit the single-strain dominance effect observed in acute NE.

4. Bacitracin and virginiamycin appear to modulate the intestinal microbiota without reducing the number and diversity of bacterial species.

5. In a clinical setting, practitioners should interpret CP susceptibility data with caution because the isolated organism may not be responsible (or fully responsible) for causing the disease. Other important factors to consider in determining a strategy for NE control include the flock’s empirical response to previous changes in management/treatment choices and prudent use of antimicrobials.

6. Further research on the characterization and/or enumeration of bacterial species comprising the poultry intestinal microbiota should lead to the development of improved practical diagnostic tools for NE.

REFERENCES AND NOTES


