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Long-Term Implications of Antibiotic Use on Gut Health and Microbiota in Populations Including Patients With Cystic Fibrosis

11

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INTRODUCTION

Since the discovery by Alexander Fleming of penicillin in 1929 and the subsequent journey of antibiotic research and discovery, antibiotics have revolutionized human health on a global scale. Antibiotic therapy can be acknowledged for a significant increase in life expectancy between 1944 and 1972, a period of intensive and successful antibiotic research (Cotter et al., 2012). However, the negative effects of antibiotic therapy are well documented, most notably antimicrobial resistance, which is now accepted as a global issue spanning medical, economic, and environmental fronts. The World Health Organization, Centers for Disease Control and Prevention (CDC),

and European Center for Disease Prevention and Control consider multidrug-resistant (MDR) infections caused by antibiotic-resistant bacteria as an emergent global disease and a major threat to public health (Roca et al., 2015). However, this review will focus on the more pervasive effects of antibiotic therapy on the gut microbiome and the far-reaching effects on the health of the host. The antibiotic-mediated perturbations of the microbiome and disruption of related functionality is an area of much research. In recent years the collateral damage caused by antibiotic use has come into focus (Blaser, 2011). Antibiotic therapy can be likened to a war of attrition on the gut microbiota because subsequent rounds of therapy result in loss of diversity followed by incomplete recovery causing the bacterial population to shift from the initial assemblage. The control of infection through antibiotic use, while not diminishing its importance, has potentially devastating consequences downstream not only for the individual, as host-microbe interactions are disturbed, but also for society with the potential dissemination of MDR determinants. In this review we examine the literature regarding the effects of various families of antibiotics on the gut microbiota, discussing in particular issues with relevance to a cystic fibrosis (CF) cohort, such as MDR as a result of chronic antibiotic use, *Clostridium difficile*, and disruption of host-microbiota mutualistic relationships.

Perturbations of the gut microbiota can occur via many external environmental factors, but perhaps the most dramatic modulatory effects are seen with antibiotic use. The immediate impact of short-term antibiotic therapy on the gut microbiota is extensively investigated. However, longitudinal studies mapping the recovery of the microbiota in the aftermath are limited, with even greater gaps in our knowledge regarding the long-term effects of chronic antibiotic use on the gut microbiota. Studies focusing on short-term antibiotic use have demonstrated the profound and pervasive effects of the antibiotic, the resilience of the gut microbiota after treatment (Dethlefsen et al., 2008; Jernberg et al., 2007), and reduction of fecal bacterial load and diversity (Xu et al., 2014; Iapichino et al., 2008; Jakobsson et al., 2010). Others have demonstrated the differential effects of different classes of antibiotics on the gut microbiota and how the resultant assembly after antibiotic treatment may be influenced by mode of action (Perez-Cobas et al., 2013). It is widely reported that immediately after antibiotic treatment an increase in *Enterococcus* species (Iapichino et al., 2008) and a decrease in *Bifidobacteria*, *Clostridia*, *Desulfovibrio*, *Faecalibacterium* and *Bacteroides* species (Adamsson et al., 1999; Bartosch et al., 2004; Jernberg et al., 2007; O'Sullivan et al., 2013) is expected. Most microbiota recover after 4 weeks; however, a long-term persistence of expressed antibiotic resistance genes, particularly carried by *Enterococcus* species, after a short-term antibiotic treatment was noted up to 4 years posttreatment in some human studies (Jakobsson et al., 2010; Jernberg et al., 2007).

CF cohorts are ideal candidate groups to investigate the effects of antibiotic use on gut health and microbiota. CF is a monogenic disorder, thereby reducing confounding factors and so facilitating inferences regarding antibiotic effects on the gut microbiota. CF is a potentially life-threatening autosomal-recessive disorder affecting 70,000 individuals worldwide, with Ireland having the highest prevalence (~1100 patients) and incidence rates (1 in 1353; Farrell, 2008). It results from mutations in

the cystic fibrosis transmembrane conductance regulator (CFTR) gene, located on the long arm of chromosome 7 (Collins, 1992), of which the most common is $\Delta F508$ (a deletion of phenylalanine at codon 508). CFTR gene mutations result in defective chloride ion transport in the respiratory, hepatobiliary, gastrointestinal, reproductive tracts, and the pancreas (Quinton, 1990). The natural progression of CF is characterized by chronic lung function decline punctuated by recurrent periods of temporary worsening of symptoms, known as pulmonary exacerbations (Amadori et al., 2009; Flume et al., 2009). Prophylactic antibiotic therapy plays a pivotal role in treating CF patients; combating frequent lung infections, slowing the rate of lung function decline, and ultimately prolonging life. Indeed antibiotic discovery, amongst other therapeutic options, has significantly contributed to the increased life expectancy of CF sufferers, meaning many survive into their thirties and beyond whereas in the 1950s few lived to attend primary school. The increasing life expectancy of CF individuals presents further new therapeutic challenges (Plant et al., 2013). A global shifting pattern of antibiotic use is seen in antibiotic therapy for CF patients in which prophylaxis, maintenance, and chronic antibiotic therapy have been shown to improve clinical outcomes and survival (Moskowitz et al., 2008; Szaff et al., 1983).

Although the gut microbiota of CF patients is poorly documented, it can be assumed that intensive and frequent courses of antibiotics alter its diversity and function as a symbiotic partner with the host. Seventy percent of the immune system is harbored in the gastrointestinal tract, meaning perturbations in the microbiome at the very least will have implications for host immunity, but also for energy extraction efficiency from food, mental well-being, and colonization resistance against pathogenic microorganisms (Weiner, 2000; Faria and Weiner, 2005; Vighi et al., 2008). Defining and characterizing the gut microbiota of a CF population can provide information applicable to the wider medical community. A picture of the gut microbiota of CF patients as a unique entity that is a signature of the disease is emerging in recent research. The characteristic CF intestinal microbiome is thought to be as a result of a combination of factors including the CFTR malfunction itself, chronic antibiotic use, high-fat diet, pancreatic enzyme supplementation, and suppression of gastric acid (Bruzzese et al., 2014). The CF gut has been shown to have lower counts of lactic acid bacteria (LAB), *Clostridium* species, *Bifidobacterium* species, *Veillonella* species, and *Bacteroides-Prevotella* species, whereas Enterobacteriaceae counts were increased in the CF cohort (Duytchaever et al., 2011). As expected in a cohort with chronic antibiotic use, CF patients have been shown to have lower species richness, evenness, diversity, and reduced temporal stability of intestinal microbiota (Scanlan et al., 2012). In particular, the reduction of diversity and abundance of *Bifidobacterium* species (*Bifidobacterium longum*, *Bifidobacterium catenulatum*, *Bifidobacterium pseudocatenulatum*, and *Bifidobacterium adolescentis*), *Clostridium* cluster XIVa, and *Clostridium* cluster IV (*R. bromii* and *Faecalibacterium prausnitzii*) has been described (Duytschaever et al., 2013). *Bifidobacterium* species have a greater antimicrobial susceptibility to many antibiotics used in the treatment of CF pulmonary exacerbations and reduced adhesion capacity to inflamed mucosa (Lee and O'Sullivan, 2010; Russell et al., 2011), perhaps explaining their reduction in a CF cohort. Evidence from Madan et al. (2012) revealed a core lung-gut microbiota

dominated by *Veillonella* and *Streptococcus*, with similarities between population fluctuations between the two sites. Furthermore, they show that select genera (*Roseburia*, *Dorea*, *Sporacetigenium*, *Coprococcus*, *Blautia*, *Enterococcus*, and *Escherichia*) of the gut microbiota precede bacterial populations found in the lung. Allelic variations of the CFTR gene have also been shown to have effects on the gut microbiota, with homozygous F508del and severe disease phenotype CF patients exhibiting greater dysbiotic fecal microbiota compared with heterozygous milder disease phenotype patients, a signature most likely reflective of reduced therapies (Schippa et al., 2013). It is hypothesized that imbalance of the intestinal microbiota of CF patients may be associated with causes, or aids, the progression of the disease and may be a predictor of expected outcomes after antibiotic therapy (Duytschaever et al., 2011).

ANTIBIOTICS USE AMONG PERSONS WITH CYSTIC FIBROSIS

Antibiotic regimens for CF patients currently follow one of the following strategies: a symptomatic regimen that is treatment of pulmonary exacerbation upon notice of acute symptoms (Elphick and Tan, 2005) or elective regimens that may comprise treatment of the chronic infection with intravenous, inhaled, and/or oral antibiotics at regular intervals to delay or prevent long-term deterioration in the absence of recent clinical deterioration (Højby, 1993; Moskowitz et al., 2008). The latter elective regimens constitute chronic antibiotic usage or prophylaxis and have become routine practice to prevent or delay pulmonary exacerbations. CF patients typically undergo antibiotic treatment involving a combination of inhaled and oral therapies as well as potentially intravenous treatments from different mechanistic classes (eg, ceftazidime and tobramycin; Smyth et al., 2014; Flume et al., 2009). With the advent of efficient nebulization devices in the 1990s, inhaled antibiotics became the mainstay in CF therapy maintenance, enabling eradication of initial *Pseudomonas* infection (Ramsey et al., 1999; Pamukcu et al., 1995; Wall et al., 1983; Moskowitz et al., 2008; Gibson et al., 2003). Inhaled antibiotics result in greater concentrations arriving directly to the respiratory system secretions, local to the infection (Moskowitz et al., 2008). Nebulized/inhaled tobramycin, aztreonam, and colistin form the main therapeutic options for eradication and/or suppression of *Pseudomonas aeruginosa* in the lung (Döring et al., 2012). Nebulization duration is associated with a significant treatment burden on the patient and may result in poorer clinical outcomes because of reduced compliance with the treatment regimen (Sawicki and Tiddens, 2012). Advances in addressing patient treatment burden are seen with the emergence of portable dry powder formulations of tobramycin (TOBI podhaler) and colistimethate sodium (Colobreathe; Konstan et al., 2011; Schuster et al., 2013; Uttley et al., 2013). Rapid delivery systems for antibiotic therapies via portable inhalers significantly reduce treatment burden and increase adherence (Harrison et al., 2014).

The success of a particular antibiotic treatment regimen is assessed primarily by considering its efficacy at suppression or eradication of the infectious agents and subsequent improvement in lung function, measured as FEV₁ (Forced Expiratory

Volume in the first second). In addition to the need for hospitalization, the length of time until the requirement for additional antibiotics, the time to reoccurrence of pulmonary exacerbation, the number and severity of adverse events experienced by patients, and the convenience and patient satisfaction (hence ease of compliance) for the patient are factors considered.

THE MODULATORY EFFECTS OF SPECIFIC ANTIBIOTICS ON THE GUT MICROBIOTA (NON-CYSTIC FIBROSIS HOSTS)

The human gut microbiota is individual specific at the bacterial strain level (Turnbaugh and Gordon, 2009; Lozupone et al., 2012; Schloissnig et al., 2013). How antibiotic therapies interact and perturb the microbiota varies between individuals, with individuals on the same antibiotic, dose, and time showing varying effects, albeit similar trends (Perez-Cobas et al., 2013). Dethlefsen et al. (2008) showed not only interindividual variation in response to ciprofloxacin but also different responses of taxa. Grouping of metagenomic data from several individuals may mask individual perturbations. Therefore it is preferable to measure distance from baseline in each individual to reveal the true effect of the antibiotic treatment (Engelbrekton et al., 2006). Diet, genetics, and health status as well as the dynamics of the microbiota itself may contribute to stability within the intestinal microbiota, enabling some communities to have more resilience than others in response to antibiotic therapy (Perez-Cobas et al., 2013). This elaborate network fed by numerous variables presents a huge challenge when analyzing microbiota responses to antibiotic treatment. Countering the effect of the antibiotic is the resilience and stability of the microbiota. The balance of the microbiota to external pressures is mediated by host–microbe interactions, microbe–microbe interactions, and physiochemical factors (Edlund et al., 2000).

The effects of antibiotics on the gut microbiome are unique to each antibiotic and depend on several factors: the target spectra, the dose and duration, the method of administration and the pharmacokinetic and pharmacodynamic properties of the agent (incomplete absorption or secretion of intravenous antibiotics by bile or intestinal mucosa may result in higher concentrations in the intestine with greater ecological effects; Edlund et al., 2000; Jernberg et al., 2010; Adamsson et al., 1999). Disturbances may be assessed (1) qualitatively (emergence of novel bacteria types with resistance genes, plasmids, and transposons) or (2) quantitatively (changes in microbiota composition due to antibiotic pressures; Edlund et al., 2000). Broad-spectrum antibiotic treatments affect not only the aberrant pathogenic bacteria but also beneficial members of the gut community, reduce diversity and richness, and disturb the ecology of the gut (Jernberg et al., 2007; Dethlefsen and Relman, 2011; O' Sullivan et al., 2012). However, it remains a challenge to distinguish antibiotic effects on the gut microbiota from other confounding factors such as stress, diet and host genetics (Jernberg et al., 2010). The route of administration has implications for antibiotic-mediated disturbances in the gut microbiota because oral, inhaled and intravenous routes result in different concentrations in different sites (Liu and Derendorf, 2003; Liu et al., 2002).

The gut microbiota has a degree of resilience to antibiotic treatment, the strength of which may vary depending on which microorganisms form a given individual's microbiota, some conferring more stability than others. Recovery of gut microbiota is often rapid, ranging from 2 days (Dethlefsen and Relman, 2011) to 2 weeks (Ubeda et al., 2010); however, individual taxa have different recovery rates, some slower than others as seen with increased proportions of Lachnospiraceae and Clostridiaceae by Antonopoulos and colleagues (2009) 6 weeks posttreatment with cefoperazone. For many, recovery is often incomplete or partial (Dethlefsen and Relman, 2011). This resilience may be apparent in the first round of therapy, and it is in subsequent, repetitive or prolonged rounds of therapy that shifts in the microbiota may be seen (Perez-Cobas et al., 2013). The magnitude and rate of the response is primarily dictated by the initial microbial community, with some communities displaying a prolonged directional shift and others a more rapid response (Dethlefsen and Relman, 2011). Initial community composition could be an important indication of expected outcomes after antibiotic use, such as likelihood of pathogen overgrowth or development of antibiotic-associated diarrhea (AAD; De La Cochetiere et al., 2008; Ozaki et al., 2004). Although the gut microbiota is stable, it is never static. Its periodic variations within a normal range make it difficult to definitively attribute loss or gain of certain taxa to certain antibiotics. Dethlefsen and Relman (2011) explain that the long-term stability of the gut microbiota is maintained by restoring forces after external disturbances rather than rigidity or resistance to change. However, the restoring forces will not withstand repeated antibiotic courses, even in cases in which the microbiota has completely recovered after an initial antibiotic treatment.

Antibiotics families vary in mode of action and target specificity and therefore in the fingerprint they leave on the gut microbiota. Perez-Cobas et al. (2013) showed that the greatest influence on the microbiota is the antimicrobial effect and the mode of antibiotic action. At the compositional level, the mode of action exerts the greatest effect, although at the functional level the antimicrobial effect is the driving force (Perez-Cobas et al., 2013). The mode of action of the antimicrobial agent produces different effects on the gut community. O'Sullivan et al. (2013) noted a significantly greater reduction of *Bifidobacterium* species and significant differences in levels of *Anaerococcus*, *Denitrobacterium*, *Faecalibacterium*, *Lactonifactor*, and *Proteus* species in subjects receiving only a nucleic acid inhibitor compared with subjects on antibiotics with cell envelope mode of action, whereas subjects receiving only cell envelope antibiotics had significantly increased levels (10-fold) of *Lactobacillus* species and significant differences in levels of *Ascomycota* and *Elusimicrobia*. They concluded that cell envelope inhibitors had less impact on the intestinal microbiota than nucleic acid synthesis inhibitors. The effects are taxa specific with some more susceptible than others; however, signatures of antimicrobial classes can be seen in bacterial profiles after treatment, with individuals' diverse microbiota converging to similar compositions when administered the same antibiotic (Perez-Cobas et al., 2013; Jernberg et al., 2010; Antonopoulos et al., 2009). The selection of antibiotic should be based not only on the expected success of the antibiotic treatment but also on the level of ecological disturbances (Edlund et al., 2000). There is a lack of information

detailing the effects of chronic usage of different antibiotic classes on the gut microbiota. Here we discuss the signatures produced by short-term use of different antibiotic classes, as shown in human studies, unless stated otherwise. See [Table 11.1](#) for a summary of the effects of specific antibiotic classes on the intestinal microbiota.

Macrolides

Macrolides are so called because of their macrocyclic ring, which may be 14, 15, or 16 membered ([Hamilton-Miller, 1992](#)). They have a protein synthesis inhibitory mode of action. Macrolides have been shown to inhibit protein synthesis by causing a dissociation of the peptidyl-tRNA from the ribosome ([Tenson et al., 2003](#)). Azithromycin has been shown to significantly improve quality of life in CF patients, reduce C-reactive protein levels, reduce the rate of lung function decline (FEV₁ measurements), reduce the number of respiratory exacerbations, and significantly reduce systemic inflammation ([Saiman et al., 2003](#); [Wolter et al., 2002](#)). Azithromycin is chosen for macrolide therapy over other macrolides, such as erythromycin, because of less adverse effects on the gastrointestinal tract. Azithromycin is also known to have negative effects on the bacterial motility of *P. aeruginosa* through suppression of flagellin expression at subinhibitory concentrations ([Kawamura-Sato et al., 2000](#)). In a study of three adults presenting with gastric and duodenal ulcers, [Jakobsson et al. \(2010\)](#) showed the negative impact of Clarithromycin on members of the Actinobacteria phyla; significant reductions of *Escherichia coli*; suppression of *Bifidobacterium*, *Lactobacillus*, and *Clostridium* species; and an increase in *Enterococcus*, *Enterobacter*, *Citrobacter*, *Klebsiella*, and *Pseudomonas* species. Clarithromycin has been shown to strongly suppress anaerobic populations in a study of healthy adults comparing Clarithromycin to a fluoroquinolone, moxifloxacin-based treatment regimen ([Adamsson et al., 1999](#); [Brismar et al., 1991](#); [Edlund et al., 2000](#)). This has important implications for total ecological disturbances because the anaerobic population is mainly responsible for colonization resistance ([Waaaj, 1989](#); [Hertz et al., 2014](#)). [Adamsson et al. \(1999\)](#) also showed a significant qualitative change with an increase of resistant *Bacteroides* from 2% to 76% in a study of 30 adults with *Helicobacter pylori* infection. This indicates an antibiotic class with the ability for ecological disruption and promotion of resistant strains. This contrasts with the findings of [Morotomi et al. \(2011\)](#), which show that macrolide antibiotic treatments tended toward dominance of *Streptococcus* species and seemed to have less dramatic effects on the microbiota with samples remaining similar to healthy intestinal microbiota in a study of 29 healthy adults ([Morotomi et al., 2011](#)).

β -Lactams

β -Lactams, encompassing cephalosporins, carbapenems, penicillins, monobactams, and cephamycins, are considered the most successful antibiotic class discovered ([Lewis, 2013](#)). β -Lactams form an important part of treatment of pulmonary exacerbation for CF patients. Ceftazidime, meropenem, flucloxacillin, piperacillin/taxobactam, and aztreonam are all β -lactam therapies commonly used by CF patients. β -Lactams have a bactericidal mode of action that disrupts cell wall synthesis,

Table 11.1 Summary of the Effects of Specific Antibiotic Classes on the Intestinal Microbiota

Antibiotic Class	Specific Antibiotic Examples	Mode of Action	Gut Microbiota Effects	References
Aminoglycosides	Tobramycin	Bactericidal agents that inhibit protein synthesis by binding the 30S prokaryotic subunit.	Little in literature. Bifidobacteria are known to have innate resistance due to lack of cytochrome-mediated transport mechanism for aminoglycoside uptake.	Bryan et al. (1979)
Macrolides	Azithromycin, erythromycin, clarithromycin	Protein synthesis inhibitory mode of action by initiating a dissociation of the peptidyl-tRNA from the ribosome.	Clarithromycin has been shown to reduce Actinobacteria phyla, <i>E. coli</i> , bifidobacteria, lactobacilli, clostridia, and anaerobic populations and increase enterococci, <i>Enterobacter</i> , <i>Citrobacter</i> , <i>Klebsiella</i> , <i>Pseudomonas</i> , and resistant <i>Bacteroides</i> .	Jakobsson et al. (2010) Adamsson et al. (1999) Brismar et al. (1991) Edlund et al. (2000)
β -Lactams	Penicillin, ceftazidime, meropenem, flucloxacillin, coamoxiclav, piperacillin/tazobactam, aztreonam	Bactericidal mode of action, disrupting cell wall synthesis, specifically in the final cross-linking stage of the peptidoglycan layer.	Increase of <i>Bacteroides</i> , <i>Blautia</i> , <i>Faecalibacterium</i> , <i>Parabacteroides</i> —most probably due to high rate of resistance in these genera. Significant reduction of anaerobic population.	Perez-Cobas et al. (2013) Monreal et al. (2005) Morotomi et al. (2011) Adamsson et al. (1999)
Lincosamides	Clindamycin	Bacteriostatic antimicrobial effect and protein synthesis mode of action.	Decreases in <i>Bacteroides</i> and <i>Blautia</i> . Increase in Enterobacteriaceae.	Perez-Cobas et al. (2013)
Fluoroquinolones	Moxifloxacin, ciprofloxacin	Inhibition of DNA gyrase and topoisomerase IV. Fluoroquinolones sequester these enzymes into a drug/enzyme/DNA complex that holds double-stranded DNA breaks together, blocking replication.	Decrease in <i>Faecalibacterium</i> , <i>Bacteroides</i> , enterococci, enterobacteria. Clostridia, Bifidobacteria, Lachnospiraceae, Ruminococcaceae, and Enterobacteriaceae.	Perez-Cobas et al. (2013) Dethlefsen and Relman (2011) Edlund et al. (2000)

Table 11.1 Summary of the Effects of Specific Antibiotic Classes on the Intestinal Microbiota—cont'd

Antibiotic Class	Specific Antibiotic Examples	Mode of Action	Gut Microbiota Effects	References
Polymyxins	Colistin sulfomethate	Induces changes to the permeability of the cell wall by binding anionic lipopolysaccharide molecules and displacing calcium and magnesium, leading to cell leakage and death.	Orally administered colistin is poorly absorbed in the gut; therefore it has little effect on the intestinal microbiota.	WHO (2006)
Nitroimidazoles	Metronidazole	Entering cell in inactive state, becoming activated in the bacterial cytoplasm through electron transfer of the nitro group of the drug to a cytotoxic state with DNA-binding capabilities.	Active against anaerobic bacteria. Decreases in Firmicutes and Bacteroidetes and increases in Enterobacteriaceae. Metronidazole concentration appears low in feces; therefore intestinal microbiota effects are minimal.	Rea et al. (2010) Sullivan et al. (2001)
Glycopeptides	Vancomycin	Interferes with glycosylation of the assembled peptidoglycan polymer after transport to the cell membrane.	Loss of <i>C. leptum</i> , <i>C. coccooides</i> , <i>C. symbiosum</i> , <i>P. luminescens</i> . Decrease in Firmicutes, Bacteroidetes, Clostridiaceae, Bacteroidaceae, Porphyromonadaceae, <i>Clostridium</i> , and <i>Odoribacter</i> . Increase in Proteobacteria, Enterobacteriaceae, Streptococcaceae, <i>Lactococcus</i> , <i>Sutterella</i> , and <i>Desulfovibrio</i> .	Rea et al. (2011) Yap et al. (2008) Murphy et al. (2013)

specifically the final cross-linking stage of the peptidoglycan layer (Page, 2012; Tipper, 1979; Strominger and Tipper, 1965). β -Lactams demonstrate broad-range activity against gram-negative and gram-positive bacteria (Holten and Onusko, 2000). Discovered in 1928 by Alexander Fleming, in 1940 penicillin was the first antibiotic to be produced in a large scale and is accredited with having saved millions of wounded during World War II. However, before its discovery the first β -lactamase was identified in 1940 in *E. coli* (Abraham and Chain, 1940), which was countered by the first β -lactamase inhibitor (clavulanic acid) in 1976. Clavulanic acid, along with amoxicillin, now forms part of Augmentin (Foulstone and Reading, 1982). After the first β -lactamase there have been many challenges to the success of β -lactams. Carbapenemases, classified as either molecular class B (metallo- β -lactamases), A, or D (serine carbapenemases, also known as oxacillinases) form three of the four known classes of β -lactamases (Miriagou et al., 2010). The host of specific carbapenemases varies depending on class. Metallo- β -lactamases are disseminated mainly in *P. aeruginosa*, but also *Acinetobacter baumannii*, *Enterobacteriaceae*, and *Klebsiella pneumonia* (Queenan and Bush, 2007, Walsh, 2008). *K. pneumonia* is the most common host of class A carbapenems (Queenan and Bush, 2007), although there have been reports of its occurrence in other species such as *Klebsiella oxytoca*, *Salmonella enterica*, *E. coli*, *Enterobacter* species, and *Pseudomonas* species (Queenan and Bush, 2007, Deshpande et al., 2006; Navon-Venezia et al., 2006; Villegas et al., 2007; Bennett et al., 2009). Oxacillinases (class D) are common in *A. baumannii* (Queenan and Bush, 2007). Most recently, the emergence of New Delhi metallo- β -lactamase-1 in 2008, which has since spread worldwide, has transformed mildly pathogenic bacteria into lethal MDR bacteria (Rolain et al., 2010). The prevalence of metallo- β -lactamases in *P. aeruginosa* is of significant clinical concern given the importance of *P. aeruginosa* in CF lung exacerbations. Metallo- β -lactamase-producing *P. aeruginosa* have shown an increase in the last 10 years and have been implicated in septicemia and pneumonia (Kouda et al., 2009; Gutiérrez et al., 2007; Pitout et al., 2007; Lagatolla et al., 2006). Extended spectrum β -lactamases (ESBLs) present another challenge to the treatment of gram-negative infections and are the main source of hospital- and community-acquired infections (Pitout and Laupland, 2008). Mainly arising in Enterobacteriaceae such as *E. coli* and *Klebsiella* species, ESBLs have plasmid encoded capability of hydrolyzing the β -lactam ring of the oxyimino-cephalosporins (cefotaxime, ceftriaxone, and ceftazidime) and monobactams (aztreonam; Bush and Jacoby, 2010; Dortet et al., 2012). Cephamycins (cefoxitin) and carbapenems (imipenem and meropenem) retain their activity against ESBL-producing bacteria. Reassuringly, in some cases β -lactamase inhibitors such as clavulanic acid and tazobactam still have the ability to inhibit ESBLs (Bush and Jacoby, 2010).

Bacteroides, *Blautia*, and *Faecalibacterium* species have been shown to increase in a study of four adults after a single antibiotic treatment with β -lactams such as ampicillin, amoxicillin, cefazolin, and sulbactam (Perez-Cobas et al., 2013; Monreal et al., 2005). This bacterial profile has been shown to be associated with bactericidal agents but not bacteriostatic antimicrobials. The increase seen in the *Bacteroides*

and *Parabacteroides* species may be explained by the presence of a high rate of β -lactamase-producing strains observed in these genera. In addition, high levels of *cfiA* gene identified suggest these strains act as reservoirs for antibiotic resistance genes and the common occurrence of *cepA* gene, which is responsible for production of cephalosporinases and penicillinases (Nakano et al., 2011; Wybo et al., 2007). Morotomi et al. (2011) also reported a dominance of *Enterococcus* genus after a single β -lactam antibiotic treatment. Adamsson et al. (1999) show that compared with a macrolide treatment (clarithromycin), a β -lactam treatment showed reduced, although still significant, ecological impact on the anaerobic populations and less emergence of resistant strains after treatment. The reduced disruption to anaerobic populations was also noted by Stark et al. (1993). In addition, Perez-Cobas et al. (2013) have demonstrated the metabolic effects of β -lactams in the alteration of bile acids, cholesterol, and hormones by intestinal bacteria. They showed a higher expression of genes related to energy metabolism during β -lactam treatment. This supports the hypothesis that long-term use of antibiotics undermines host-microbe mutualistic relationships.

Lincosamides

Clindamycin is a class of lincosamide antibiotics used to treat mainly anaerobic infections (Hedberg and Nord, 2002) with a bacteriostatic antimicrobial effect and a protein synthesis inhibitor mode of action. Resistance to clindamycin has increased significantly over the last two decades. Perez-Cobas et al. (2013) observed marked decreases in *Bacteroides* and *Blautia* genera immediately after administration; however, after 3 days a recovery of *Bacteroides* was observed, suggesting acquisition of resistance genes by these bacteria. They also noted that clindamycin induced an increase in Enterobacteriaceae compensating for loss of anaerobic bacteria. In comparison with other agents (fluoroquinolone or β -lactams) clindamycin was shown to result in a more variable and stronger effect on microbial community structure. Clindamycin has been shown to result in an increase in abundance of resistance genes, relative to other antibiotics such as moxifloxacin, amoxicillin, and cefazolin/ampicillin/sulbactam combination, mostly of the efflux pump class (Perez-Cobas et al., 2013).

Fluoroquinolones

Fluoroquinolones inhibit bacterial cell proliferation by inhibiting DNA gyrase and topoisomerase IV, both essential enzymes in bacterial DNA transcription and replication (Hooper, 2001). In addition to inhibiting these two enzymes, fluoroquinolones sequester these enzymes into a drug/enzyme/DNA complex that holds double-stranded DNA breaks together, blocking replication (Drlica, 1999). Treatment with different antibiotics from the fluoroquinolone class presents a similar initial response in the gut microbiota. Moxifloxacin showed a decrease in *Faecalibacterium* and *Bacteroides*, with ciprofloxacin showing similar effects with additional decreases seen in Lachnospiraceae and Ruminococcaceae (Perez-Cobas et al., 2013; Dethlefsen and Relman, 2011). Earlier fluoroquinolones such as ciprofloxacin, levofloxacin,

norofloxacin, and ofloxacin target aerobic bacteria, mainly gram negatives, but similar to moxifloxacin, a methoxyquinolone, they result in a reduction in numbers of Enterobacteriaceae. Moxifloxacin has a wider reaching activity than the others with activity against respiratory pathogens, including β -lactamase-producing aerobic gram-positive cocci; *Haemophilus* and *Moraxella*; aerobic gram negatives; and intracellular and atypical microorganism such as *Legionella*, *Chlamydia*, and *Mycobacterium*. Moxifloxacin also has activity against gram-positive and gram-negative anaerobes. In a study looking at the effects of repeated courses of oral ciprofloxacin (500 mg twice daily for 5 days at 2- and 8-month time points in a 10-month study) and the disturbance to the gut microbiota in three subjects, [Dethlefsen and Relman \(2011\)](#) showed an almost complete return to the pre-ciprofloxacin state after the first treatment; however, after a second treatment the microbiota community composition was different from what it had been at the start of the study and appeared to be stable in its new state. [Edlund et al. \(2000\)](#) also showed significant decreases of enterococci, enterobacteria, bifidobacteria, and clostridia during moxifloxacin treatment (400 mg oral administration once daily for 7 days).

An interesting feature of fluoroquinolones is their ability to bind reversibly to bacteria and fecal material, resulting in reduced amounts of the drug in the intestine, translating to less disturbance of the total microbial intestinal population by fluoroquinolones ([Edlund et al., 2000](#); [Lidbeck et al., 1988](#)). Because of this, moxifloxacin has a more targeted effect on the gut microbiota with more limited ecological disturbances ([Edlund et al., 2000](#)).

Polmyxins

Colistin sulfomethate is cyclopeptide, belonging to the family of polymyxins, which is produced by *Bacillus polymyxa* var. *colistinus* ([Jeong et al., 2009](#); [Storm et al., 1977](#)). Colistin is mainly active against gram-negative organisms. Its mode of action involves inducing changes in the permeability of the cell wall by binding anionic lipopolysaccharide molecules and displacing calcium and magnesium, thus causing cell leakage and death ([Jeong et al., 2009](#)). Orally administered colistin is known to be poorly absorbed in the gastrointestinal tract, and studies show that when excreted it is bound to the fecal material ([Eichenwald and McCracken, 1978](#)). This may reduce the perturbation effect of the drug in vivo. [Jeong et al. \(2009\)](#) show that *E. coli* was the most susceptible to colistin. Colistin sulfomethate is one of the most frequently used antibiotics for treatment of exacerbations in CF patients. Early eradication of *Pseudomonas* infection with nebulized drug, aggressive treatment of acute exacerbations with intravenous therapy, and long-term suppressive maintenance therapy again nebulized are the three primary modes of usage in CF management. Colistin forms part of each of these ([Littlewood et al., 2000](#)). *P. aeruginosa* is known to frequently develop resistance to β -lactam antibiotics and aminoglycosides. However, thus far in vitro resistance of *Pseudomonas* species to colistin is rare, cementing its place as a useful treatment for pseudomonas infections for the foreseeable future. [Wright et al. \(2013\)](#) have shown using in vitro culture methods that antibiotics (ceftazadime, colistin, azithromycin, and

tobramycin) at subinhibitory concentrations result in the phenotypic population diversification of *P. aeruginosa*. It is interesting to note that they demonstrated that ceftazidime and colistin contributed to more diversification than tobramycin and azithromycin. Diversification of the *P. aeruginosa* populations in the CF lung negatively affect antibiotic therapy success, leading to a chronic infection state and ultimately to progression of the disease. Whether this diversification pressure is also exerted on taxa in the gut has not been reported. Knowledge of differential diversification potential of antibiotics can contribute to more informed decisions for antibiotic selection in management and control of infection.

Nitrimidazoles

Metronidazole is the prototype of the nitroimidazole family and most widely used for treatment of anaerobic and protozoal infections (Lamp et al., 1999). Along with fidomoxacin and vancomycin, metronidazole is the mainstay of *C. difficile* treatment (Surawicz et al., 2013; Cornely et al., 2014), estimated to have colonized up to 50% of CF patients (Binkovitz et al., 1999; Bauer et al., 2014). Metronidazole is known to have activity against gram-negative (*Bacteroides fragilis*) and gram-positive (*C. difficile*) anaerobes (Lofmark et al., 2010). Metronidazole functions by entering the cell in its inactive or prodrug state, becoming activated in the bacterial cytoplasm through an electron transfer to the nitro group of the drug, or protozoa organelles, to a cytotoxic state with DNA-binding capabilities. Inhibition of DNA synthesis and DNA damage by oxidation leads to DNA degradation and cell death (Land and Johnson, 1999; Diniz et al., 2000; Lofmark et al., 2010). Its activity is limited to anaerobic bacteria because aerobes lack the appropriate transfer proteins, conferring them with intrinsic resistance to metronidazole (Reysset, 1996). Mechanisms of resistance to metronidazole are primarily based on either decreased uptake of the prodrug or reduced electron transfer capabilities (Land and Johnson, 1999). Nitroimidazole (*nim*) resistance genes encode an alternative reductase protein that converts nitroimidazole to a nontoxic derivative, thus avoiding DNA damage (Reysset, 1996; Leiros et al., 2004; Lofmark et al., 2010). Metronidazole resistance is generally low, although there have been reports of resistance in *Sutterella* species (Jousimies-Somer et al., 2002). *C. difficile* maintains its susceptibility to metronidazole with no significant clinical resistance observed (Lofmark et al., 2010; Terhes et al., 2014). In a study using high-throughput sequencing, Rea et al. (2011) demonstrated phylum (reduction of Firmicutes and Bacteroidetes and increase of Proteobacteria) and family (increasing Enterobacteriaceae) effects of administering metronidazole in a colonic model experiment. The concentration of metronidazole in feces is low as a result of efficient absorption; therefore alterations to the normal microbiota after metronidazole treatment are minimal (Sullivan et al., 2001). Nagy and Foldes (1991) propose a mechanism of inactivation of metronidazole by intestinal enterococci, thus reducing its effect on the gastrointestinal microbiota. The success of oral metronidazole in *Clostridium difficile* infection (CDI) treatment is a result of the elevated plasma serum levels that diffuse through the damaged intestinal mucosa of infected individuals with greater efficiency (Sullivan et al., 2001).

Glycopeptides

Glycopeptide antibiotics have an inhibitory mode of action on late-stage peptidoglycan polymer synthesis. Glycopeptide antibiotics interfere with glycosylation of the assembled peptidoglycan polymer after transport to the cell membrane (Reynolds, 1989). Vancomycin, teicoplanin, and most recently telvancin are the only glycopeptide antibiotics approved by the US Food and Drug Administration (FDA; Van Bambeke et al., 2004, Damodaran and Madhan, 2011). Glycopeptides are active against most gram-positive organisms but few gram-negative organisms. Vancomycin remains the first-line drug against methicillin-resistant *Staphylococcus aureus* (MRSA); however, emergence of resistance in enterococci and staphylococci has led to a restriction in the use of vancomycin as well as teicoplanin (Van Bambeke et al., 2004; Damodaran and Madhan, 2011). Yap and colleagues showed the disturbance effect of vancomycin on the gut microbiota in mice using a 2×100 mg/kg per day dose for 2 days. *Clostridium leptum*, *Clostridium coccoides*, *Cenarchaeum symbiosum*, and *Photorhabdus luminescens* were lost immediately after treatment, with subsequent rapid recovery of *C. leptum* and *C. coccoides*. In an environmentally controlled 24-h model distal colonic experiment, Rea et al. (2011) showed a decrease in the major phyla, Firmicutes and Bacteroidetes, and an increase in Proteobacteria after addition of 90 μ M vancomycin at eight hourly intervals. The rapid proliferation of Enterobacteriaceae at the expense of other gram negatives and Firmicutes during vancomycin treatment is reported (Rea et al., 2011; Yap et al., 2008). In a diet-induced obesity mouse model administering 2 mg/day vancomycin for 20 weeks, Murphy et al. (2013) attributed the decrease in Firmicutes and Bacteroidetes at the phylum level to a decrease in Clostridaceae and Bacteroidaceae at the family level, respectively, and increasing Proteobacteriaceae was attributed to increasing Enterobacteriaceae. At the family level they also noted decreases in Porphyromonadaeae and increases in Streptococcaceae and Desulfovibrionaceae, whereas at the genus level increases in *Lactococcus*, *Sutterella*, and *Desulfovibrio* and decreases in *Clostridium* and *Odoribacter* were observed.

Effects of Combination Therapy on Gut Microbiota

Combination or multidrug therapy often confers advantages over single drug therapy in terms of a wider range of targets, synergistic effects, reduction of MDR organisms, and reduction in total amount of antibiotic used; however, it incurs high costs, complications for administration, and drug-related toxicity (Elphick and Tan, 2005; Perron et al., 2012; Gould and van der Meer, 2007). Taylor et al. (1993) reported that 80% of clinics had preference for combination therapy. Combinational antibiotic therapy poses challenges to identifying, quantifying, and comparing the specific effects of antibiotic classes on the gut microbiota. At present, treatment of *P. aeruginosa* infection in patients with CF involves two to three antibiotics, demonstrating improved synergy (Zebouh et al., 2008; Hewer and Smyth, 2014). In general, this may include the use of inhaled antibiotics such as tobramycin and colistin (Littlewood and Macdonald, 1987, Ratjen et al., 2001), oral quinolones such as ciprofloxacin (Taccetti et al., 2005), and intravenous antibiotics usually involving a β -lactam antibiotic in combination

with an aminoglycoside antibiotic (Döring et al., 2000, Hewer and Smyth, 2014). Combination therapies are a commonly used approach for biofilm management, with the rationale that multiple antibiotics, with a range of individual targets can combine to help suppress or eradicate the varying antagonistic phenotypes present in the biofilm (Barraud et al., 2013). Colistin is known to be active against bacterial cells with low metabolic activity whereas tetracycline and ciprofloxacin are known to be active against metabolically active cells (Pamp et al., 2008; Herrman et al., 2010).

Conclusive results yielding specific guidelines for treatment of pulmonary exacerbations and delaying chronic *P. aeruginosa* infection in CF patients have yet to be reached on whether combinational or monotherapy is more effective because of a lack of studies testing combinational therapy options versus monotherapy in a head-to-head manner with control for confounding factors. Of the studies available, it is difficult to unequivocally indicate which is the most effective because studies differ in length of administration of antibiotic, age, health status and history of patient cohort; differing times from detection to initiation of treatment; frequency of surveillance; markers of success; and statistical analysis. However, the current physician consensus infers a preference for combinational therapy for patients with CF (Elphick and Tan, 2005).

LONG-TERM ISSUES OF CHRONIC ANTIBIOTIC USE

MULTIDRUG RESISTANCE

Almost immediately after the introduction of the first antibiotics, a parallel emergence of resistance has inhibited their success (Davies and Davies, 2010). Most recently, a World Health Organization report on antimicrobial resistance (AMR) (2014) warns of the dangers of entering a postantibiotic era in which minor infections could emerge as life threatening diseases in the face of an AMR pandemic. MDR is a major public health issue facing the medical and wider community (Laxminarayan et al., 2013). In the European Union (EU), AMR is estimated to be responsible for 25,000 deaths each year, whereas health-care-related costs are estimated to be €0.9 billion with a further €1.5 billion expenditure on productivity losses (Rodier, 2011; Report EEJT, 2009). MDR is compromising our ability to manage infectious disease. Well-documented MDR organisms include *P. aeruginosa* and *Burkholderia cepacia* complex, as well as emerging methicillin-resistant *Stenotrophomonas maltophilia* and MRSA. MDR was responsible for 23,000 deaths in the United States in 2013, of which MRSA and *Streptococcus pneumoniae* were responsible for 11,000 and 7000 deaths, respectively (CDC, 2013). Antibiotic overuse and misuse is the driving force of the development of resistant organisms (Laxminarayan and Heymann, 2012). An empirical strategy of treatment based on expert consensus remains the mode of action on diagnosis of pulmonary exacerbation. This generalized treatment strategy inevitably contributes to the adverse effects of antibiotic overuse in terms of dose and time. This is compounded by the increase in age of the CF demographic

(Waters and Ratjen, 2006) globally (CFRI Annual report 2011; Davis, 2006; www.cff.org), with the ratio of adult CF patients to pediatrics increasing each year.

The human gastrointestinal tract is an environment where 10^{13} – 10^{14} microorganisms are in constant interaction, providing an ideal situation for rapid dissemination of resistance genes between commensals and pathogens (Salyers et al., 2004; Davies, 1994). The human gut microbiota is now viewed as a reservoir where commensals and pathogens are in direct contact, thus facilitating the spread of resistance genes (Salyers et al., 2004; Sommer et al., 2009; Fouhy et al., 2014). Studies have established that the gut resistome is established in infancy, even in the absence of antibiotic therapy, growing and diversifying with age (Fouhy et al., 2014). In response to antibiotic therapy, resistance genes are expressed and selected, with strains carrying resistance proliferating, amplifying the resistance reservoir (Lu et al., 2014; Fouhy et al., 2014). In CF patients, or other groups on chronic antibiotic therapy, the resistome is likely to have increased capabilities. Whole genome sequencing has shown that lateral gene transfer and mobilizable elements that incorporate into various host bacteria are responsible for most resistance (Ochman et al., 2000). Transferable resistance genes may arise in commensals and through lateral gene transfer mechanisms spread to clinically relevant pathogenic strains. It is postulated that the gut microbiome may facilitate the acquisition of resistance by pathogenic strains (Sommer et al., 2009). Sommer et al. (2009) used a functional metagenomic approach to characterize the antibiotic resistome of two healthy unrelated humans who had not been treated with antibiotics for a least 1 year. Phylogenetic profiling of aerobically cultured strains from the gut microbiome showed they were primarily members of the Proteobacteria phyla and to a lesser extent Firmicutes and Actinobacteria. Resistance genes belonging to one of the following classes—tetracycline efflux pumps, two classes of aminoglycoside-modifying enzymes, and three classes of β -lactam-inactivating enzymes (TEM, AmpC, and CTX-M)—were detected. Characterizing the human gut resistome may increase understanding of the origin of the antibiotic resistance, ultimately addressing the MDR issue.

The expression and subsequent persistence of resistance genes ensuing antibiotic treatment varies depending on antibiotic class, duration, and dose strength of treatment. Persistence of resistance may be observed years after an antibiotic course. For instance, after a 5-day antibiotic treatment (500 mg twice daily) with ciprofloxacin, initial gut microbiota perturbations recover; however, resistance to the particular antibiotic is enriched and may be observed for months or even years afterwards (Dethlefsen et al., 2008). Likewise, Sjölund et al. (2003) showed that in three of five patients post-clarithromycin treatment, erythromycin resistance was present after 1 year and in one patient after 3 years. Jernberg et al. (2007) also showed the persistence of resistance genes (*ermF*, *ermG*, and *ermB*) after clindamycin treatment up to 2 years after a 7-day antibiotic treatment. Shoemaker et al. (2001) demonstrated that the presence of the *tetQ* gene has increased from 30% to 80% in all strains of *Bacteroides* as a result of conjugal gene transfer in the last three decades.

Some genera may contribute to multidrug resistance more than others by acting as a reservoir for mobilizable resistance determinants. *Bacteroides* and *Parabacteroides* species are mutualistic inhabitants of the gut performing many beneficial

functions for the host, such as carbohydrate metabolism (Xu et al., 2003). *Bacteroides* form 25% of the adult human gut microbiota (Salyers, 1984) and have been implicated in many opportunistic infections, particularly *B. fragilis* (Wexler, 2007; Cao et al., 2014). They have an innate ability to produce β -lactamases such as cephalosporinases and penicillinases encoded by the *cepA* gene (Gutacker et al., 2000). Clindamycin resistance, encoded by the *ermF*, *ermB* or *ermG* genes or through efflux pumps, in *Bacteriodales* ranges from 10% to 42% and confers protection against macrolides, lincosamides, and streptogramin B (Wybo et al., 2007; Gupta et al., 2003; Nakano et al., 2011; Shoemaker et al., 2001; Pumbwe et al., 2007). In a study by Nakano and colleagues (2011), 53.5% of tested *Bacteroides* and *Parabacteroides* strains were tetracycline resistant, primarily because of the acquisition of the *tetQ* ribosome protection protein. The rise of resistance genes in predominating and functionally vital genera of the human gastrointestinal tract provide pathogens with increased opportunity for acquisition of many resistance determinants.

AMR rates are increasing and studies such as the SMART (Study for Monitoring Antimicrobial Resistance Trends), PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin), and SENTRY (an international, longitudinal study on the susceptibility of pathogens sponsored by Bristol Meyers Squibb) seek to globally monitor and characterize AMR rates (Morrissey et al., 2013). The SMART study describes the increase in prevalence of ESBLs, conferring resistance to β -lactams, in Asia, Europe, Latin America, Middle East, North America, and the South Pacific. The study also reports the increasing resistance to fluoroquinolones, particularly in *P. aeruginosa* (increases from 22% to 33% were seen in North America). Macrolide resistance rates have also been shown to be increasing (Cresti et al., 2002). Recent studies have indicated this is due to the epidemic spread of *ermB* resistance genes among streptococcal populations and in particular *Streptococcus pyrogenes*, either by horizontal gene transfer or clonal expansion of resistant isolates after selective pressures of antibiotic treatment (Jakobsson et al., 2010). Sjölund et al. (2003) have reported the presence of macrolide-resistant enterococci after selection by clarithromycin and metronidazole.

Development of Opportunistic Pathogens and Antibiotic-Associated Diarrhea

Chronic antibiotic therapy results in significant alterations in the gut microbiota, creating opportunities for opportunistic pathogens, primarily *C. difficile*, to establish and proliferate. *C. difficile* is mostly a nosocomial infection of the elderly, but it can also be community acquired, affecting younger populations and adults. The CDC has linked *C. difficile* to 14,000 deaths in America (CDC, 2013). The European Center for Disease Control estimates that CDI costs the EU €3 billion per year (ECDC, 2015). Broad-spectrum antibiotic therapy has been associated with increased incidence of *C. difficile* and is identified as one of the most well-recognized risk factors for *C. difficile* and indeed recurrent and refractory CDI (Bartlett and Gerding, 2008; Diggs and Surawicz, 2009). Broad-spectrum antibiotics induce a dysbiotic gut microbiota, particularly among anaerobic populations, providing *C. difficile* with an opportunity to proliferate. Indeed, Owens et al. (2008) reported 94% of hospitalized patients with

CDI had received antibiotics before or during their hospital stay. *C. difficile* is responsible for 20–25% of AAD cases and 90–100% of pseudomembranous colitis (Rea et al., 2010; Cramer et al., 2008). Ironically, antibiotic treatment of CDI perpetuates its proliferation, increasing strains resistant to vancomycin, particularly enterococci (Chang et al., 2008), lowering diversity and providing *C. difficile* with opportunity to proliferate. The association between ampicillin, cephalosporins, and fluoroquinolones and CDI is well recognized in the literature (Gerding, 2004; Sullivan et al., 2001). It is interesting to note that the emergence of particular antibiotics as risk factors for CDI has paralleled the frequency of that particular antibiotic class in general medical use. The increase of CDI was initially associated with clindamycin, which is the preferential treatment for various anaerobic, streptococcal, and *Staphylococcus* infections. Since the 1990s, the increase in the use of fluoroquinolones to treat various infections has precipitated these antibiotics in becoming risk factors for CDI and has contributed to the emergence of hypervirulent strains such as ribotype 027, which is characterized by fluoroquinolone resistance, binary toxin production, and increased toxin production due to a mutation in the *tcdC* gene (Pepin et al., 2005; Kuijper et al., 2008). Metronidazole and vancomycin have also been determined as risk factors for CDI. In cases in which antibiotics are necessary to treat concomitant infection, alternative antibiotics to those identified as CDI development risk factors should be used where possible. As expected, increased duration of antibiotic courses and antibiotic combination therapy are associated with increased risk of CDI (Owens et al., 2008). Not all antibiotics for CDI infection are associated with increased risk. Piperacillin in combination with tazobactam has shown considerable success in treatment of CDI but with less associated risk of increased development of infection or relapse (Owen et al., 2008). Vancomycin and, more recently, fidaxomicin (2011) are the only drugs approved by the FDA for CDI treatment. Fidaxomicin is particularly effective in treating recurrent CDI because it has less impact on the fecal microbiota, translating into a more resilient microbial assemblage after treatment. Fidaxomicin shows less risk of development of resistance than vancomycin in *in vitro* studies (Zar et al., 2007). Alternative therapies that may facilitate the reduction of antibiotics are necessary to treat CDI. These include fecal microbial transplant (FMT), which is an alternative therapy at conceptual stage with early pioneering case studies showing considerable success in patients with reoccurring symptomatic *C. difficile* (Petrof et al., 2013; Koenigskecht and Young, 2013). Another alternative includes thuricin CD, a novel antimicrobial peptide (AMP) or bacteriocin produced by *Bacillus thuringiensis*, which has been patented as a CDI therapy (Rea et al., 2010). The potency of thuricin CD has been shown to be greater than vancomycin and in some cases greater than metronidazole. In addition, thuricin CD shows no antimicrobial activity against a range of commercially available probiotics, demonstrating its narrow substrate range (Rea et al., 2011).

Asymptomatic carriage of *C. difficile* in CF is well recognized; however, the mechanisms of protection against infection, but not colonization, are not fully understood. Carriage rates in healthy adults vary from 0% to 15% (Viscidi et al., 1981; Nakamura et al., 1981), compared with up to 50% in CF cohorts—a result of prophylaxis and frequent hospitalizations. However, unlike normal populations in which

approximately 50% of colonized individuals develop CDI, or in more severe cases pseudomembranous colitis, development of response to *C. difficile* toxins is rare in CF patients (Kyne et al., 2000; Peach et al., 1986; Welkon et al., 1985). It is hypothesized that the unique microbial assemblage of CF patients with high levels of Enterobacteriaceae, lactobacilli, *Pseudomonas*, and *Staphylococcus* confers protection and resistance against *C. difficile* growth, but not colonization (Rolfe et al., 1981). Welkon et al. (1985) suggest an altered gut pH as a result of defective ion transport inhibits toxin production or degrades toxins, although there is no evidence to support this. Another possibility postulates that exposure to *C. difficile* at such an early age mediates an immune response that protects in later life (Welkon et al., 1985). The relatively low incidence of CDI despite high rates of toxigenic *C. difficile* colonization and antibiotic usage is an area of much needed research.

Metagenomic studies in cohorts with *Clostridium difficile*-associated diarrhea (CDAD) that have investigated microbiome shifts noted increases in *C. difficile* and Bacteroidetes, *C. coccoides*, *Eubacterium rectale*, *Ruminococcus gnavus*, and *Clostridium nexile* (Chang et al., 2008). It is interesting to note that the Bacteroidetes phylum seems to show a strong association with CDAD in several studies. Manges et al. (2010) show that low levels of Bacteroidetes are associated with CDAD. Tvede and Rask-Madsen (1989) demonstrate using bacteriotherapy experiments that the absence of *Bacteroides* species such as *Bacteroides ovatus*, *Bacteroides vulgaricus*, and *Bacteroides thetaiotaomicron* may result in recurring CDI whereas their abundance in the gut microbiota may afford protection against colonization and proliferation against *C. difficile*. Low Firmicutes and *Bacteroides* ratios, along with increasing facultative anaerobes, have been shown to be associated with *C. difficile* colonization in infants (Rousseau et al., 2011). Metagenomic studies of patients with CDI noted a decrease in alpha diversity at the DNA level (Chang et al., 2008; Knecht et al., 2014). However, Knecht et al. hypothesize that instead of a microbiota-wide compositional change, a loss of functionality of a single species may impair the resilience of the microbiota to *C. difficile* colonization. These functions may include short-chain fatty acids (SCFAs) or antimicrobial production, which may protect against *C. difficile* proliferation. Recent research by Knecht et al. (2014) indicates that Lachnospiraceae may be one such species in which low or high levels are associated with protection against or colonization with *C. difficile*.

Antibiotic substitutions for other options with less risk of CDI development associated may be effective (Vonberg et al., 2008). Further research into drug development is warranted to provide options that cause less disruption to the microbiota, consequently affording *C. difficile* less opportunity to proliferate.

Interference of Mutualistic Host–Microbe Interactions through Antibiotic-Induced Alteration of Gut Microbiome

The human intestinal microbiota develops a partnership with the host and is essential to health and functions such as nutrition, immunoregulation, metabolism, development, and pathogen resistance. With approximately 100 times more genes than the human genome, the gut microbiome lends us functional features we have not had to

evolve ourselves (Backhed et al., 2005). Although the intestinal community does seem to have a level of taxonomic functional redundancy, antibiotic-mediated elimination or significant reduction of important members of the microbiota is likely to affect functionality and host physiology, particularly in the case of chronic antibiotic therapy (Perez-Cobas et al., 2013). Many key species of the human intestinal microbiota perform important functions; however, disruption of the core gut colonizers by antibiotic therapy may have a more profound effect on the functioning of the microbiota as a symbiotic partner of the host. Examples include *B. thetaiotaomicron*, a prominent mutualist in the intestinal microbiome, which has a genome-encoded capacity for carbohydrate metabolism far greater than the carbohydrate-metabolizing capabilities of our human genome (Shipman et al., 2000; Backhed et al., 2005). The gut microbiota also functions in regulation of bile acid and choline metabolism. The gut microbiota ecosystem comprises a dynamic network of functional interactions. As in any ecosystem, even targeted, or narrow-spectrum antibiotic effects will influence not only the target but also indirectly affect many interaction partners (Willing et al., 2011). Antibiotic therapy can induce host immunity effects through the loss of bacterial ligands, metabolites, and specific bacterial signals (Willing et al., 2011). Receptors involved in host immunity, such as toll-like receptors and NOD-like receptors are activated by specific bacterial ligands such as lipopolysaccharide, lipoteichoic acid, flagellin, and peptidoglycan. The loss of specific bacterial ligands reduces immune signals (Wells et al., 2011). Metabolic profiles of antibiotic-treated mice show a reduction of SCFAs, with downstream reduction in the antiinflammatory effects of SCFAs as well as functions in regulation of cell proliferation, differentiation, growth, apoptosis, vasodilation, and wound healing (Yap et al., 2008; Millard et al., 2002; Leung et al., 2009; Bergman, 1990). Populations of bacteria have specific signals associated with them that may be lost after antibiotic treatment. In addition, many studies report reduced expression and secretion of AMPs, directly affecting first-line microbial defense (Meyer-Hoffert et al., 2008). Taken together, these factors result in a host more susceptible to pathogen insult (Willing et al., 2011).

The diversity of the human gut microbiota is the result of natural selection at the microbial level and the host level (Backhed et al., 2005). Diversity confers resilience to stress, such as antibiotic therapy, by harboring a diverse range of responses. This is known as the insurance hypothesis (Guarner, 2007). In general, low diversity correlates with poor health status (Lozupone et al., 2012; Claesson et al., 2012). This is one of the primary caveats of gut health and is demonstrated in infants, adults, and elderly populations. Many disease states have been associated with low-diversity microbiota signatures in recent years. Antibiotics as agents drive the microbiota to an altered assemblage through a gradual drift or dramatic shift, which may lead to the onset of microbiota-related diseases associated with less diversity, such as obesity (Turnbaugh et al., 2006), diabetes (Larsen et al., 2010), and inflammatory bowel disease (IBD; Dicksved et al., 2008). Turnbaugh et al. (2006) demonstrated that colonization of germ-free mice with “obese” microbiota resulted in development of obese phenotype and likewise in lean mice. Childhood antibiotic use and the resulting altered microbiota are potentially associated with asthma, atopic disease and obesity

(Marra et al., 2006; Noverr and Huffnagle, 2005). Low gut microbiota diversity in infants has been associated with eczema (Abrahamsson et al., 2012), particularly of the phylum Bacteroidetes and its genus *Bacteroides*. Indeed, in a study of 123 CF children, 59% presented with skin hypersensitivity allergy (Warner et al., 1976). Although not proposed by the authors at the time, low-diversity gut microbiota may have contributed to the hypersensitivity observed. The reduction of gut microbiota diversity after exposure to antibiotics is well documented. Antibiotic exposure may reduce overall diversity and/or diversity of select taxonomic groups, as seen by Jernberg et al. (2007) in a study of four patients exposed to 150 mg clindamycin 4 times daily. They observed an overall reduction in gut microbiota diversity but also a reduction in diversity of the *Bacteroides* community and emergence of clindamycin-resistant strains. In a study investigating the effects of ciprofloxacin (500 mg twice daily for 5 days) on the gut microbiota of adult patients, the abundance of one-third of bacterial taxa were reduced (Dethlefsen and Relman, 2011). Likewise, Rea et al. (2010) showed reduction of diversity after exposure to metronidazole and vancomycin in a human colonic model, mostly accounted for by the proportional increase of Enterobacteriaceae. However, despite the well-acknowledged effect of the lack of diversity on host health, the functional redundancy and resilience of the gut microbiota is also accepted. Indeed, Dethlefsen et al. (2008) have demonstrated that although the gut microbiota was disturbed after treatment with ciprofloxacin, they observed a continuity of microbial functions. It remains to be investigated whether the low-diversity microbiota disorders described could be the result of perturbations brought about by antibiotics alone or a combination of factors in which antibiotic therapy is perhaps the factor that tips the balance to a disease state.

The hygiene hypothesis is quoted frequently as an argument against sterilizing our environment and indeed our bodies through antibiotic use (Wills-Karp et al., 2001). The increasing incidence of autoimmune disorders such as asthma, eczema, celiac disease, and IBD may be related to disruption of normal host–microbe interactions by antibiotics (Guarner et al., 2006). Guarner hypothesizes that deficient exposure to mutualistic bacteria such as bifidobacteria and lactobacilli explains the increase of immunodysregulatory disorders in modern society. Guarner proposes the use of prebiotics and probiotics as a more favorable method of infection control.

MODULATION OF THE GUT MICROBIOTA DIET

Diet has rapid and profound modulatory effects on the gut microbiota composition and functionality. Various diet types, including high-fat, high-sugar, “Western,” and low-fat, polysaccharide-rich diets, have been associated with microbiota signatures differing at the compositional and functional gene level. Particular bacterial species have genetically encoded specific metabolic capabilities. The gut microbiota adapts to dietary intake to select bacterial populations with the genetic capabilities best suited

for metabolism substrates of a given diet (Scott et al., 2008; Power et al., 2014). The rapid response of the gut microbiota to changes in diet has been demonstrated by studies in conventionalized germ-free mice. A change from a low-fat/plant-rich diet to a high fat and sugar/low plant polysaccharides “Western diet” showed changes in the gut microbiota within a single day, with increases in Firmicutes and decreases in Bacteroidetes (Turnbaugh et al., 2009). Hildebrandt et al. (2009) showed a similar rapid response in the gut microbiota to a dietary change (high-fat/low-fiber or low-fat/high-fiber) within 24 h. Most pertinent to CF are the effects of a high-fat diet on the gut microbiota composition. Dietary requirements for CF persons include 120–150% estimated average requirement of energy and 200% reference nutrient intake for protein (Littlewood and Macdonald., 1987; Pencharz and Durie., 1993). In addition, it is recommended that the CF total calorie intake should comprise 40% fat (White et al., 2004). The response of the gut microbiota to dietary fat is well documented. De Filippo et al. (2010) established the profound difference between the gut microbiota of European children on a typical Western diet (high fat, sugar, animal protein, and starch and low fiber content) and children in the African state of Burkina Faso, who consumed a predominantly vegetarian diet that was high in plant polysaccharides, starch, and fiber and low in animal protein and sugar. The Burkina Faso children had a lower abundance of Firmicutes and a higher abundance of Bacteroidetes genera (*Xylanibacter* and *Prevotella*), involving cellulose and xylan hydrolysis, enabling efficient energy extraction from their plant-rich diet. Indeed, a study by Wu et al. (2011) linked gut microbial enterotypes dominated by either *Bacteroides* or *Prevotella* with diets dominated by either protein and animal fat or carbohydrates along with low meat and dairy intake, respectively. A high-fat diet has been shown to influence a less diverse microbiota and a higher proportion of Bacteroidetes, *Parabacteroides*, *Eubacterium*, *Anaerotruncus*, *Lactonifactor*, and *Coprobaillus* in elderly subjects (Claesson et al., 2012). In the same study, community-dwelling subjects having a predominantly low to moderate fat intake had higher abundance of *Prevotella*, *Coprococcus*, and *Roseburia*. In addition to compositional microbial effects, a high-fat diet has been shown to result in a low-grade intestinal inflammatory state, which is a hallmark of CF (Arkan et al., 2005; Werlin et al., 2010).

Gastrointestinal manifestations of CF include exocrine pancreatic insufficiency (PI), steatorrhea, intestinal inflammation, distal intestinal obstruction syndrome (DIOS) and cystic fibrosis–related diabetes (CFRD). PI occurs in 90% of CF patients, resulting in malabsorption of fat; protein; and fat-soluble vitamins A, D, E, and K and subsequent malnutrition (Fieker et al., 2011; Dodge and Turck, 2006). Undigested, unabsorbed dietary fat is excreted in stools, known as steatorrhea (Somaraju and Solis-Moya, 2014). PI predisposes CF individuals to the onset of DIOS, an obstruction of fecal transit at the ileocecal junction (Somaraju and Solis-Moya, 2014). DIOS occurs in 10–20% of patients because of increased water absorption as a result of defective intestinal CFTR. CFRD occurs in approximately 2% of children, 19% of adolescents, and 40–50% of adults presenting with CF. CFRD, distinct from the type I and type II diabetes found in the general population (Stecenko and Moran, 2010) is associated with poorer pulmonary function and nutritional status (Koch et al., 2001;

Marshall et al., 2005). Although there is a paucity of information on how these gastrointestinal manifestations affect the intestinal microbiota, especially in the CF context, a microbiota response to such comorbidities is probable.

Positive Modulation of the Gut Microbiota

Positive modulation of the gut microbiota and host health can be achieved using probiotics, most commonly *Lactobacillus* or *Bifidobacterium* species. The beneficial effects of probiotics are perhaps more subtle than previously thought. Scientists are moving from a theory that probiotics alter total gut microbiome composition to a concept of prebiotics conferring a direct health benefit on the host (Power et al., 2014; Ouwehand et al., 2002). Thus far probiotics have been demonstrated to have therapeutic potential in treatment of AAD (Vidlock and Cremonini, 2012; Hempel et al., 2012), CDI (Na and Kelly, 2011), IBD (Isaacs and Herfarth, 2008), and irritable bowel syndrome (Andersen and Baumgart, 2006). There have been preliminary but promising studies trialing administration of *Lactobacillus rhamnosus* GG (LGG) as a probiotic in pediatric CF cohorts. Bruzzese et al. (2004, 2014) showed the success of LGG in reducing intestinal inflammation as indicated by the noninvasive inflammatory markers nitric oxide and fecal calprotectin. The treated cohort also reported decreased abdominal pain. To further investigate the positive effects of LGG for the CF population, Bruzzese et al. (2007) investigated the effects of LGG administration on the rate of pulmonary exacerbation. They demonstrated a protective effect of LGG in children chronically infected with *Pseudomonas* and consequently a reduction in pulmonary exacerbations and increase in FEV₁. Weiss et al. (2010) showed a significant reduction in pulmonary exacerbations in children with CF infected with *P. aeruginosa* after administration of a probiotic cocktail containing *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus*; 90% of patients were also receiving azithromycin treatment at the time. The offsite effects of *Lactobacillus* species on *Pseudomonas* in the lung suggest a direct effect between the former and the latter, supported by earlier studies in mice in which LGG administration reduced *Pseudomonas* species, and *Lactobacillus plantarum* showed inhibitory activity toward *Pseudomonas* species (Valdez et al., 2005). Bruzzese et al. (2014) showed partial recovery of gut microbiota in CF children on antibiotic treatment who were subsequently treated with LGG with a significant increase of *Bacteroides* and a trend toward an increase in *F. prausnitzii*, a known antiinflammatory agent.

Probiotic strains with antibiotic tolerance may have application after or during antibiotic therapy. However, antibiotic tolerance in probiotic bacteria is generally associated with safety concerns by food safety authorities such as the European Food Safety Authority (EFSA) because of the risk of dissemination of resistance determinants from probiotics to commensal or pathogenic members of the gastrointestinal microbiota. Plasmid or mobile genetic element mediated resistance carried by many probiotic genera poses a serious safety issue (Sharma and Singh Saharan, 2014). In addition, there are issues for antibiotic–probiotic combination therapy and possible resulting antagonistic relationships that may exist. Increased bacterial load due to the presence of viable probiotics may reduce the potency of antibiotics. It has been shown that

the potency of vancomycin decreases depending on the numbers of bacteria exposed (Udekwu et al., 2009). Probiotic-antibiotic combinations that preserve the potency of the antibiotic and the viability of the probiotic must be selected (Hammad and Shimamoto, 2010). Antibiotic-tolerant probiotic therapies remain at the conceptual stage because of difficulty in determining safety. However, the potential for application in a cohort with chronic antibiotic usage warrants further research into this novel area.

Knowledge of which microbiota community composition resists change more efficiently may be important in probiotic development. It may be possible to direct microbial communities toward a more resilient assemblage in individuals for whom chronic antibiotic use is necessary. In the future, probiotics may provide an adjunct therapy along with antibiotics for the treatment of CF symptoms. The possibility of reduction of antibiotics through use of alternative therapies, such as probiotics, is attractive to patients and clinicians.

FUTURE DIRECTIONS

Antimicrobial discovery has been in decline since the 1970s, and MDR is an increasingly serious threat to global public health. As an immediate response to MDR we can curb the inappropriate use and overuse of antibiotics, gain knowledge on antibiotic effects, and administer at doses that target aberrant bacteria while beneficial bacteria are preserved, all of which can help preserve and maintain the effectiveness of antibiotics for the next generation. In this age of high-throughput, next-generation sequencing technologies, it is imperative that we use the technologies available to us to tailor the antibiotics currently available to the individual instead of the “catch-all” broad-spectrum approach that has consequences on our antibiotic resources. CFMATTERS (Cystic Fibrosis Microbiome Derived Antimicrobial Therapy Trial in Exacerbations Results Stratified) is an EU-funded multicenter study currently examining this question in adult patients with CF (www.cfmatters.eu, <https://clinicaltrials.gov/ct2/show/NCT02526004>). Alternative additional strategies dealing with infection need to be brought from the bench to mainstream clinical practice. There have been several studies investigating the use of bacteriophage as a therapeutic to treat CF lung infection, particularly in treating *Pseudomonas* infection, but also *S. aureus* and *B. cepacia* (Brussow, 2012; Debarbieux et al., 2010; Alemayehu et al., 2012; Morello et al., 2011). Phage therapy is advantageous over antibiotic therapy because of its specificity to the target pathogen, its ability to replicate at the site of infection, and its ability to evolve along with bacterial populations that may develop resistance. Alemayehu et al. (2012) have demonstrated the efficacy of a phage cocktail (a myovirus (ϕ NH-4) and a podovirus (ϕ MR299-2)) to clear murine *Pseudomonas* lung infection. Furthermore, it has been shown that phage not only cleared *Pseudomonas* infection but also prevented infection when administered 24 h before infection, highlighting a potential role of phage in prophylaxis (Debarbieux et al., 2010; Morello et al., 2011).

Inhibition of *Pseudomonas* virulence and biofilm formation through disabling quorum sensing networks is another potential therapeutic approach for infection

control. O'Loughlin et al. (2013) have demonstrated the utility of a quorum sensing inhibitory molecule (meta-bromo-thiolactone) to block pathogenesis by interacting with the two major quorum sensing systems Lux I/R. In addition they demonstrated the potential for preventative and curative treatment using meta-bromo-thiolactone. Further work is needed in these areas.

CONCLUSION

The collateral consequences of antibiotic therapy on the gut microbiota have been widely studied, and it is accepted that chronic and/or long-term antibiotic use has compositional and potentially functional effects on the gut microbiota. In a cohort with chronic antibiotic usage, such as CF, antibiotic resistance capabilities are expected to be high with consequences for subsequent antibiotic therapy. The long-term persistence of resistance genes in the gut microbiota in response to antibiotic therapy poses a concern for the clinical management of infection. Moving forward, tailored, individualized antibiotic strategies may prove critical along with additional options other than antibiotics. Phage therapy and targeting of quorum sensing networks may provide alternatives or adjunct therapies to antibiotic therapy in the future. Probiotics may be used as restorative agents to reintroduce depleted populations and maintain the gut microbiota ecosystem. In the interim, the medical community and wider society must endeavor to preserve our antibiotic resources through strategic and rational administration.

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