Impact of antimicrobial therapy on the gut microbiome

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The gut microbiome is now considered an organ unto itself and plays an important role in health maintenance and recovery from critical illness. The commensal organisms responsible for the framework of the gut microbiome are valuable in protection against disease and various physiological tasks. Critical illness and the associated interventions have a detrimental impact on the microbiome. While antimicrobials are one of the fundamental and often life-saving modalities in septic patients, they can also pave the way for subsequent harm because of the resulting damage to the gut microbiome. Contributing to many of the non-specific signs and symptoms of sepsis, the balance between the overuse of antimicrobials and the clinical need in these situations is often difficult to delineate. Given the potency of antimicrobials utilized to treat septic patients, the effects on the gut microbiome are often rapid and long-lasting, in which case full recovery may never be observed. The overgrowth of opportunistic pathogens is of significant concern as they can lead to infections that become increasingly difficult to treat. Continued research to understand the disturbances within the gut microbiome of critically ill patients and their outcomes is essential to help develop future therapies to circumvent damage to, or restore, the microbiome. In this review, we discuss the impact of the antimicrobials often used for the treatment of sepsis on the gut microbiota.

Introduction

The gut microbiota—the microbes that collectively inhabit the human intestine—plays a key role in protecting the body against potentially harmful entities such as bacteria, toxins and antigens.[1] The interaction between sepsis and the microbiota can be regarded as a so-far incompletely understood bidirectional relationship. The disease state of sepsis has a disruptive effect on the microbiota, but the interventions during clinical care for these critically ill patients are external modulators of the microbiota as well.[2]

The rapid administration of appropriate antimicrobial therapy to patients with sepsis is vital and associated with both lower in-hospital and 30 day mortality when compared with inappropriate empirical antibiotics.[3,4] The initial treatment of sepsis critically influences the clinical outcome for the patient. Empirical therapy regimens in critical illness often consist of multiple, broad-spectrum antimicrobial agents to ensure appropriate coverage of potential pathogens of concern. However, antibiotics are no longer considered only beneficial; they may also be potentially harmful agents, as multiple studies have shown that their use can have severe and long-lasting effects on the composition of the microbiota. With earlier recognition of the aetiology of infection it is possible to limit the harm to the microbiota by reducing unwarranted antimicrobial exposure.[5–7]

The duration of antimicrobial therapy has been independently associated with the development of *Clostridioides* (*Clostridium*) *difficile* infection (CDI).[8,9] Additionally, numerous studies have demonstrated the correlation of antimicrobial exposure with the impact on colonization and drug-resistant pathogens.[10–13] For example, the proliferation of VRE after antimicrobial exposure is also of concern as the high bacterial burden increases the risk of dissemination via translocation and can subsequently lead to bloodstream infections. The antibiotic-mediated depletion of commensal bacteria decreases intestinal RegIII-γ expression, which normally acts to resist colonization by VRE.[14] Furthermore, these disruptions in the microbiota may predispose a patient to recurrent infection and sepsis.[15,16] Not surprisingly, microbiota-targeted therapies are being developed to prevent or treat sepsis.[17,18] It remains to be seen to what extent these changes in the microbiota influence the clinical outcome of those who suffer from sepsis. One of the many challenges to understanding the association between antimicrobial administration and the effects on the microbiota in patients with sepsis is the varying level of antimicrobial exposure hospitalized patients receive, which is difficult to capture.[10–12] The spectrum of activity, dose received, route of administration, and

the pharmacokinetic and pharmacodynamic properties of the antibiotic agent will all determine the extent of its effect on the microbiota.[5,6] In addition, numerous other treatments given to patients with sepsis, such as proton pump inhibitors, enteral/parenteral feeding, anti-inflammatory drugs, sedatives, opioids and catecholamines, have all been described to impact the gut microbiota.[18,19] The effects of medication on the microbiome remain significantly underexplored, as demonstrated by recent *in vitro* screen testing of 1200 marketed drugs, which found 50% of non-bacterial anti-infectives and 25% of all human-targeted drugs inhibit at least one gut commensal.[19]

As evidence continues to support a prime role of the microbiome in sepsis, knowledge on the interaction of the host and the causative microorganism, as well as the ecological impact of antimicrobial agents, is of great clinical importance. In this review, we briefly discuss the effect of sepsis and antibiotics utilized during sepsis on the composition of the gut microbiota.

Effects of the disease state of sepsis on the gut microbiome

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection.[20] Several studies have shown a loss of diversity of the microbiome—the collection of all genomes of microbes in an ecosystem—in critically ill patients.[1,2,21–23] The loss of diversity, called dysbiosis, has been described to be potentially associated with poor outcome, although the underlying mechanism still needs to be elucidated.[21,24] Ojima *et al.*[25] found that extremes in the ratio of Bacteroidetes relative to Firmicutes in stool samples was predictive of death compared with survival.[2] Furthermore, the relative abundance of *Pseudomonas aeruginosa* in the microbiota of endotracheal tubes was predictive of patient survival, with survivors favouring the phylum Actinobacteria, which includes bifidobacteria, usually found in probiotic therapies.[26] The microbiome of patients in the ICU is characterized by a loss of diversity, site specificity and microbial richness, as well as overgrowth of opportunistic pathogens, usually tending towards a single taxon.[2,21,27,28] Depletions in *Faecalibacterium*, which produces short-chain fatty acids vital for a healthy gut, were also seen.[29] There are large interpersonal differences in microbiome dysregulation, which can be expected as critically ill patients are continuously exposed to a wide range of endogenous alterations that have been shown to modulate the composition of the gut microbiota (e.g. an increased production of catecholamines, altered glucose metabolism and gastrointestinal dysmotility).[18,21,30]

It is difficult to disentangle the effect of sepsis itself on the microbiota and the effect of the treatment given for sepsis. A murine study of pneumonia-derived sepsis in which mice were inoculated intranasally with the Gram-negative bacillus *Burkholderia pseudomallei* showed a marked shift in faecal bacterial composition in all septic mice, with a strong increase in Proteobacteria and decrease in Actinobacteria.[31] These results are in line with recent reports on intestinal dysbiosis in mice inoculated via the airways with influenza as well as *Mycobacterium tuberculosis*.[32,33] In these studies, no *B. pseudomallei*, influenza or *M. tuberculosis* was detected in faeces. These studies demonstrate that the systemic inflammatory response itself can lead to marked alterations in the gut microbiota during sepsis.

Neonatal sepsis and microbiome

Premature or low birth weight neonates and infants often receive multiple courses of antibiotics for conditions such as culture-negative sepsis or prophylaxis, which, together with their low-diversity microbiome, may lead to profound long-term health consequences (e.g. asthma, psoriasis, other autoimmune diseases and obesity/metabolic imbalance), as well as susceptibility to the development of infectious diseases.[34–38] Additionally, through antibiotic pressure, putatively beneficial commensal bacteria may be replaced by MDR pathogens. These factors pose a threat to the otherwise quasi-stable microbiome that develops by around age 3 years.[39]

Regardless of class, overall increased antibiotic selective pressure is associated with reduced bacterial diversity and colonization with MDR organisms. Studies report a 2- to 9-fold increased risk of MDR bacterial acquisition among patients treated with antibiotics, which also leads to enhanced shedding and hence infectivity.[40] However, some antibiotics affect species richness more than others.[41] In addition to other confounders (e.g. breastfeeding, overall health), treatment with meropenem, cefotaxime or ticarcillin/clavulanate is associated with significant reduction in species richness.[42,43] This relationship between antibiotics and species richness is now the target of focused research. Studies in premature infants have also reported a proposed association between specific potential pathogens and an increased risk of necrotizing enterocolitis (NEC). Increased abundance of Enterobacteriaceae (shift towards Proteobacteria) together with reduced endogenous anaerobes and reduced microbial diversity preceding NEC onset support these findings.[44] More than 5 days of antimicrobial therapy for suspected early-onset sepsis is associated with an increased risk of developing NEC and overall mortality.[42]

Effects of interventions during sepsis on the microbiome

The most detrimental effect on the microbiome is potentially caused by the widespread use of antibiotics.[45] A prospective, multicentre, point-prevalence study (1 day) collecting data in 1265 ICUs worldwide showed that 71% of all patients received antibiotics.[46] Even macrolides that are sometimes utilized as prokinetics, notwithstanding direct antimicrobial effects on the microbiome, cause alterations in gut transit time, which have been shown to have a comparatively large effect size, accounting for ~5% of observed compositional variation.[47] As outlined above, aside from antimicrobial treatment, there are additional effects of non-antibiotic treatment for sepsis (or critical illness) on the microbiome, such as the impact of proton pump inhibitors and nutritional support.[17]

The clinical relevance of dysbiosis of the microbiome in sepsis remains poorly defined. However, this pathobiome appears highly unfavourable, with potential links to sepsis-induced immunosuppression.[17] There are some strategies to modulate the microbiome during critical illness, such as the use of selective decontamination of the digestive tract (SDD) to prevent pathological overgrowth.[48,49] Despite some data on SDD demonstrating a reduction in lower airway bloodstream infection, this has not translated into widespread clinical practice.[50] This may partly be due to fears of the development of MDR bacteria; this is unfounded, based on the majority of studies, with the caveat that they have largely been performed in ICUs with low baseline levels of antimicrobial

resistance. Buelow *et al.*[23] showed that in a small cohort, SDD only led to the selection of four resistance genes and concluded that the risks associated with antibiotic resistance are limited. However, there was evidence to suggest that recolonization with MDR bacteria may occur on ICU discharge and cessation of SDD. Lastly, given the complexity of the data around SDD, the findings are often difficult to interpret and some studies have suggested that SDD is not associated with benefits, as recently demonstrated.[51,52]

Probiotics are already used as therapy in some ICUs, potentially decreasing the incidence of ventilator-associated pneumonia.[53] A recent randomized synbiotic trial suggested they could prevent sepsis in neonates in India.[54] Approximately 4500 healthy newborns were randomized to receive a 7 day course of either placebo or oral synbiotic preparation (*Lactobacillus plantarum* in fructooligosaccharide, chosen based on pre-clinical data showing superior gut colonization). The trial was terminated early after a 40% risk reduction for the primary outcome (death or sepsis) was shown in the treatment arm. What is more intriguing is the concomitant reduction in lower respiratory tract and skin and soft tissue site infections, suggesting a more systemic benefit of gut microbiome modulation.

Effect of antimicrobial therapy on the gut microbiome

The impact of commonly used antimicrobial therapies on the gut microbiota is discussed here, starting with a β-lactam/β-lactamase inhibitor combination, cephalosporins, carbapenems and fluoroquinolones. Table 1 provides a summary of the faecal data after administration of antimicrobials discussed in this review.[55]

Piperacillin/tazobactam

Piperacillin/tazobactam is an empirical antimicrobial agent utilized in hospitalized patients for an array of suspected infections caused by Gram-negative organisms. The addition of the β-lactamase inhibitor tazobactam extensively expands the spectrum of the parent compound, allowing broad coverage of potential pathogens of concern. Nord and colleagues[56] investigated the effects of piperacillin/tazobactam on the human microbiota in patients receiving the agent for 4–8 days for the treatment of intra-abdominal infections. Although the overgrowth of organisms such as *C. difficile* was not observed, presumably due to the agent's inhibitory activity against the organism, decreases in anaerobic organisms such as bifidobacteria, Eubacteria and lactobacilli were observed.[56,57] While only minor decreases in enterococci were observed during treatment, upon discontinuation of treatment with piperacillin/tazobactam, faecal concentrations of enterococci increased to levels that surpassed the pretreatment faecal concentrations.[56] Of greater concern was the isolation of resistant *Enterobacter* spp., which were discovered in three patients as a result of the treatment, potentially a result of de-repressed AmpC production in the presence of drug pressure. As faecal microbiota data suggest the development of resistance in Enterobacteriaceae following exposure to piperacillin/tazobactam, this was confirmed in a randomized clinical study in patients with intra-abdominal infections treated with either piperacillin/tazobactam or ertapenem.[11] In comparison with patients treated with ertapenem, those treated with piperacillin/tazobactam had a significantly higher rate of resistant

Enterobacteriaceae identified with rectal swabs. Another study evaluated the effect of piperacillin alone on the colonic microflora in 20 patients undergoing colorectal surgery who were treated with the agent for 2 days of prophylaxis.[58] Decreases in anaerobic cocci were observed as described in the above studies. Two out of 20 patients had *C. difficile* isolated with the presence of toxin production. Importantly, both patients were noted to be on prior antimicrobial therapy. Despite only receiving piperacillin therapy for 2 days, the disruptions of the intestinal microflora did not return to pre-treatment patterns for up to 4 weeks.[58]

Although piperacillin/tazobactam has not been associated with VRE colonization in murine models, clinical findings in patients in the ICU have demonstrated otherwise.[59,60] In a study of 146 patients with documented VRE-negative rectal swabs prior to therapy, new colonization rates of VRE observed in patients treated with cefepime compared with those treated with piperacillin/tazobactam were similar, ranging from 25% to 30%.[60] While piperacillin/tazobactam is not generally considered to have a strong association with VRE when compared with other antibiotics (e.g. extended-spectrum cephalosporins), studies evaluating this association often categorize the class of penicillins together. The challenge with this generalization is the varying extent of biliary excretion and anaerobic activity within the class, which ultimately determines the disrupting effect of these agents on the microbiome ecology.

Cefepime and ceftazidime

Cefepime is a fourth-generation cephalosporin clinically administered for its broad-spectrum activity against Gram-negative pathogens, including *P. aeruginosa*, and for its stability against AmpC-producing organisms. The effects of cefepime on the intestinal flora were studied in 12 healthy individuals in a placebo-controlled study to assess the agent's impact on the faecal microflora. Decreases in the number of *Escherichia coli* and bifidobacteria in faeces were observed, as well as minimal increases in *Bacteroides* spp. and *C. difficile*.[61] Additionally, after 8 days of cefepime treatment it took 20–48 days for faecal bacteria to normalize.

Ceftazidime is a third-generation cephalosporin, also with broad-spectrum Gram-negative activity, including activity against *P. aeruginosa*. The impact of ceftazidime on the faecal microbiome was studied in eight healthy volunteers. As expected, the intestinal Enterobacteriaceae content was suppressed at the end of the treatment course.[62,63] Intestinal anaerobic organisms such as *Lactobacillus bifidus* had a slight decrease and *Bacteroides fragilis* had minimal fluctuations overall. The observation of ampicillin- and cefazolin-resistant Enterobacteriaceae 2 weeks after the last dose was the most significant influence of ceftazidime on the intestinal flora. Resistance to ceftazidime was not observed.[62] Similar findings were observed in a study performed by Knothe and colleagues.[63]

Cephalosporins, as a class, have been previously associated with VRE colonization.[64,65] However, the link between individual antibiotics within the class of cephalosporins and VRE colonization has not been well described until recently.[66] An association specifically between days of cefepime/ceftazidime therapy per 1000 patient days and incident VRE colonization was observed. However, this observation did not persist in multivariate analysis controlling for demographic and clinical covariates. It is challenging to obtain

Table 1. Summary of antimicrobials and their effects on the faecal microbiota[a]

Antimicrobial class	Antimicrobial	Effects on faecal microbiota			Reference
		decrease	increase	stable	
Penicillins	piperacillin/tazobactam	Enterobacteriaceae bifidobacteria Eubacteria lactobacilli		*Bacteroides* enterococci clostridia	56
Cephalosporins	cefepime	*E. coli* bifidobacteria		*Bacteroides* clostridia	61
	ceftazidime	Enterobacteriaceae lactobacilli		enterococci *Bacteroides*	62, 63
	ceftriaxone	Enterobacteriaceae *E. coli* lactobacilli bifidobacteria clostridia	enterococci	*Bacteroides*	72, 73
Carbapenems	meropenem	Enterobacteriaceae clostridia *Bacteroides*	enterococci	yeast lactobacilli bifidobacteria Eubacteria	83
	imipenem	Enterobacteriaceae enterococci bifidobacteria Eubacteria lactobacilli *Bacteroides*		clostridia	85
	ertapenem	*E. coli* bifidobacteria *Bacteroides*	enterococci	lactobacilli clostridia	86
Fluoroquinolones	ciprofloxacin	Enterobacteriaceae	enterococci	anaerobic flora bifidobacteria	90, 91
	levofloxacin	*E. coli* enterococci clostridia		bifidobacteria lactobacilli *Bacteroides*	92
	moxifloxacin	*E. coli* enterococci bifidobacteria clostridia		lactobacilli *Bacteroides* fusobacteria	94

[a]Vancomycin not discussed due to minimal effects of intravenous vancomycin on the gut.[55]

clinical data on the risks and incidence of colonization with resistant organisms due to multiple confounders. Observational changes in hospital-acquired infection rates can also be observed when drug shortages result in an increased use of another class of antimicrobials as a substitution.[67] VRE rates were found to double during a national piperacillin/tazobactam shortage that resulted in a substantial increase in cefepime use at the institution.

Ceftriaxone

Ceftriaxone is a third-generation cephalosporin with pseudomonal-sparing Gram-negative activity. The extensive biliary penetration of this agent makes it unique compared with other β-lactams and also makes it a preferred agent for the treatment of certain infections,

such as cholecystitis.[68] However, this pharmacological property is also thought to result in greater disturbances of the gut microbiota. The effects of ceftriaxone on the intestinal microbiota have been studied in various patient populations, including healthy volunteers as well as acutely infected patients.[69–72] These studies found that Enterobacteriaceae in the gut were either largely suppressed or eliminated. While decreased levels of anaerobic bacteria were also found in most of these studies, they were not suppressed to the extent of Enterobacteriaceae. Additionally, overgrowth of *Enterococcus* spp. was observed. Interestingly, the data from Nilsson-Ehle and colleagues[73] suggest a correlation between biliary clearance of ceftriaxone and the impact on the gut microbiota, given that the two patients with a minimal impact on aerobic bacteria also had the lowest biliary clearance. Furthermore, the one patient that had

toxin-producing *C. difficile* in the faeces during and after treatment with the presence of diarrhoea had the highest biliary clearance.

The effect of third-generation cephalosporins on faecal flora was also studied in children aged between 2 and 18 months that received antimicrobial therapy for serious bacterial infections.[74] Pre-treatment stool specimens revealed uniform and normal intestinal flora, with *Pseudomonas* spp. and *Candida albicans* present in small quantities. The first dose of ceftriaxone resulted in elimination of susceptible aerobic bacteria from the faeces within only 48 h. Additionally, the specimens showed increasing presence of *C. albicans* and enterococci until Gram-negative flora reappeared. During treatment, the most common Gram-negative organism isolated was *P. aeruginosa.*

To better understand the emergence of resistance, de Lastours and colleagues[75] compared stool samples of patients receiving ceftriaxone with those of patients that were not and found that ~27% of patients receiving ceftriaxone had a novel AmpC-overproducing Enterobacteriaceae. While there was a slight increase in ESBL organism colonization, this rate was not different from that in hospitalized patients who did not receive antibiotics, possibly due to the risk of transmission of ESBL organisms in the hospital setting regardless of antibiotic exposure. Persistence of colonization with AmpC-producing organisms was discovered in three out of four patients at the long-term follow-up conducted between 3 and 6 months post-treatment. A correlation between specific ceftriaxone pharmacokinetic concentrations and amplification of CTX-M resistance genes has also been previously described. An fC_{max} of >30 mg/L or $fAUC_{0-24}$ >222 mg·h/L was associated with an increased risk of amplification.[76] Additionally, a treatment duration of >14 days, irrespective of drug exposure, was associated with an increased risk of amplification.

Ceftriaxone's prolonged half-life allows the drug to be administered once a day, making it an attractive treatment choice for a wide variety of inpatient and outpatient infections, but this also results in higher use compared with other cephalosporins.[77] The risk associated with colonization by resistant Gram-negative bacilli as a result of ceftriaxone use has been described in the literature.[75,76,78,79] A study from a single institution analysing antibiotic consumption and CDI found a significant clinical correlation between ceftriaxone use and healthcare facility onset CDI.[80] Similar conclusions were derived from a meta-analysis performed using 14 studies that included predominantly case–control observational studies. This meta-analysis indicated that the use of third-generation cephalosporins was associated with the highest risk of hospital-acquired CDI.[81] Like other cephalosporins, ceftriaxone usage has been associated with VRE. One study showed that ceftriaxone use was related to nosocomial VRE bloodstream infection incidence while other antimicrobials, such as piperacillin/tazobactam, ceftazidime and cefepime, did not exhibit the same correlation.[82]

Despite these data, ceftriaxone is often perceived as a de-escalation agent from standard empirical agents such as piperacillin/tazobactam or cefepime. While the agent has a narrower spectrum of activity compared with advanced-generation cephalosporins or extended-spectrum penicillins, the known effects on the gut microbiome, the data identifying the risk of colonization of resistant pathogens and the extensive accumulation in the gastrointestinal tract are concerning.

Carbapenems

With reliable activity against ESBL-producing pathogens, carbapenems are often used as empirical therapy in patients with a history of, or at high risk of, a drug-resistant organism. Although changes in the intestinal microflora have been observed in patients receiving meropenem and imipenem, they were deemed relatively minor compared with those caused by other carbapenems, presumably due to very low concentrations of both agents observed in the faeces.[83–85] Ertapenem, however, was shown to have a larger impact on the intestinal microflora and has been recovered in more substantial concentrations in the faeces. When studied in healthy volunteers, ertapenem was shown to decrease anaerobic organisms such as *Bacteroides* spp.[86] Decreases in *E. coli* and increases in enterococci, similar to those seen with ceftriaxone, were also noted.[86] Notably, these studies were in healthy volunteers or patients receiving short courses of therapy for surgical prophylaxis, and therefore the duration of drug exposure and the host response were likely not comparable to the duration infected patients would have experienced.

The impact of ertapenem on the acquisition of resistant Enterobacteriaceae and VRE has also been compared with that of piperacillin/tazobactam in patients treated for intra-abdominal infections.[11] Although not statistically significant, rectal swabs identified more patients in the ertapenem group (6.4%) who acquired VRE compared with the piperacillin/tazobactam group (1.6%). However, patients treated with ertapenem had lower rates of resistant Enterobacteriaceae.

Despite less significant and more variable changes in the faecal microbiome with imipenem and meropenem, both agents have been associated with concerning clinical implications regarding the risk of acquiring resistant pathogens. Tacconelli and colleagues[10] evaluated the relationship between antibiotic therapy and the acquisition of resistant pathogens, particularly MRSA, VRE and ciprofloxacin-resistant *P. aeruginosa*. These data showed that carbapenems, specifically imipenem and meropenem, were associated with the highest risk of antibiotic-resistant bacteria, with 14 new cases identified per 1000 antibiotic days. A meta-analysis of randomized studies investigating the risk of CDI associated with antibiotics found that carbapenems were associated with a higher risk than cephalosporins and even fluoroquinolones, the latter being recognized as predisposing patients to CDI.[87] While this finding is rather unique to this meta-analysis, it is noteworthy that when a subgroup analysis evaluating the class of carbapenems without ertapenem was performed, a similar CDI occurrence was observed. Furthermore, this meta-analysis was different in that only randomized studies were evaluated, whereas other analyses included observational studies.[88]

Fluoroquinolones

Their excellent oral bioavailability and potent *in vitro* activity make fluoroquinolones commonly administered agents for a variety of conditions, including sepsis derived from the urinary or respiratory tract. Despite significant toxicity concerns (e.g. tendon rupture, hypoglycaemia, neuropathy), the ease of transitioning to an oral equivalent has contributed to the overuse of these agents, such that from 2000 to 2010 there was a 64% increase in prescribing.[89] Unfortunately, this overuse has also led to the emergence of fluoroquinolone resistance, which has negative consequences for

clinical outcomes. The effects on faecal microbiota of commonly used fluoroquinolones such as ciprofloxacin, levofloxacin and moxifloxacin have all been studied, with relatively consistent results.[90–94] As expected, all of these studies demonstrated significant decreases in faecal concentrations of Enterobacteriaceae, many of which observed complete eradication with concentrations below the limit of detection. To a lesser and more variable extent dependent on the individual fluoroquinolone, anaerobic bacteria (e.g. clostridia, bifidobacteria and Bacteroides spp.) were affected. The emergence of fluoroquinolone-resistant E. coli is perhaps one of the most clinically significant consequences of fluoroquinolones for the microbiome. Despite profound elimination of Enterobacteriaceae, several studies have documented the emergence of these resistant E. coli strains as a result of fluoroquinolone treatment.[95] The rate of fluoroquinolone-resistant E. coli was found to be ~15%–20% in a study of 451 hospitalized patients receiving a course of fluoroquinolones.[96] Neither the duration of therapy nor the type of fluoroquinolone received was found to have a strong association with acquiring drug-resistant E. coli. Arguably, the most concerning and often forgotten element from this data set is the 5.6% rate of horizontal transfer of resistant strains to patients not receiving fluoroquinolones but housed in the same ward.[96] Furthermore, resistance mutations that target biological functions of a cell can sometimes result in a fitness cost to the organism. However, this was observed neither in fluoroquinolone-resistant E. coli nor in the more recently problematic ST131 fluoroquinolone strains that are not only found in hospitalized patients but also colonize the gut of healthy subjects.[97]

The connection between the use of fluoroquinolones and CDI has been demonstrated in clinical studies.[88,98] However, this link was not as profound in the previously mentioned meta-analysis that evaluated only randomized studies.[87] The relationship between fluoroquinolone use and CDI seems to be strongly associated with the BI/NAP1/027 strain, which, although initially isolated in North America, has spread to other parts of the world.[99] Therefore, it is possible that the studies alluding to less significant associations between fluoroquinolones and CDI had overall lower use of fluoroquinolones and subsequently lower rates of fluoroquinolone-resistant C. difficile strains.

New agents

Studies of the effect of newer broad-spectrum antimicrobial agents (e.g. ceftaroline, ceftobiprole, telavancin and tigecycline) on the human microflora of healthy volunteers were recently reviewed by Rashid and colleagues.[100] Ceftaroline, ceftobiprole and telavancin were all found to have minor ecological effects on the intestinal microbiota following 7 days of antibiotic administration. Furthermore, no new colonizing aerobic or anaerobic bacteria resistant to these agents were observed. The effect of tigecycline on the intestinal microflora was more extensive.[101] Reductions in the number of enterococci, E. coli, lactobacilli and bifidobacteria (but no impact on Bacteroides spp.) were observed in the intestinal microflora, while numbers of other Enterobacteriaceae and yeasts increased. Additionally, tigecycline-resistant strains of Enterobacter cloacae and Klebsiella pneumoniae were recovered in some patients. Most intestinal microbiota disruptions returned to normal by the end of the 31 day study period.

In recent years, three β-lactam/β-lactamase inhibitor combinations have come to market: ceftazidime/avibactam, ceftolozane/tazobactam and meropenem/vaborbactam. Rashid and colleagues[102] investigated the effect of ceftazidime/avibactam on the intestinal microflora of healthy volunteers and found that it had a significant ecological impact on the intestinal microbiota. The number of E. coli and other Enterobacteriaceae decreased significantly during the administration of ceftazidime/avibactam, whereas the number of enterococci increased. Lactobacilli, bifidobacteria, clostridia and Bacteroides spp. decreased significantly during ceftazidime/avibactam administration. Toxigenic C. difficile strains were detected in 5 of the 12 volunteers during the study. The impact of ceftolozane/tazobactam and meropenem/vaborbactam on intestinal flora has not yet been described in published literature.

Time to disruption

Although the faecal microbiota data discussed here support the idea that even short courses of antibiotics can cause significant disruption to the gut microbiota, it is much more challenging to determine exactly when this disruption occurs. Reasons for this challenge include interpatient variability and our understanding of what changes are clinically meaningful, as opposed to arbitrary findings. Studies that examine the gut microbiome using molecular methods provide a more comprehensive evaluation of sequential changes in the gut microbiome.

The faecal microbiota of a 39-year-old patient receiving amoxicillin/clavulanic acid at 875/125 mg twice daily for 10 days for acute sinusitis was studied.[103] The patient developed loose stools within 24 h of therapy. Stools were collected from the first daily bowel movement during antibiotic therapy and on a weekly basis thereafter. The day 0 sample was composed mainly of Bacteroides spp., Clostridioides rRNA clusters IV and XIVa, and Bifidobacterium spp. In just 4 days after the start of therapy, there was a shift in the composition of the microbiota that included elimination of the Clostridioides rRNA cluster XIVa and Bifidobacterium spp. Additionally, 34% of the sequences were of Enterobacteriaceae, which originally represented only 2% of sequences on day 0. With the exception of bifidobacteria, normalization of the microbiota was observed by day 24. Despite the patient's diarrhoea not being associated with C. difficile, as toxin production was not present, it was clear that the gut microbiota was greatly affected. In another study, De La Cochetière and colleagues[104] used molecular methods to analyse the faecal microbiota of six volunteers receiving a 5 day course of amoxicillin. The profiles were compared among each other on the basis of similarity, with day 0 being used as a standard reference. In the four volunteers that provided daily stool samples, similarity percentages decreased to an average of 73% on day 3, ranging from 62% to 82%. Moreover, while the average on day 4 remained at 74%, the range expanded to 46%–94%, signifying the magnitude of interpatient variability.

Although less frequently studied, the long-term effects of antibiotics on the microbiome have been described.[105,106] One study evaluated the effects of a 10 day course of ciprofloxacin on the microbiome over the course of a year, with faecal samples collected a total of six times.[106] Using culture-based techniques, a decrease in the number of Gram-negative aerobes was observed, consistent with the results of the studies

described above. However, when molecular techniques were utilized, the impact of ciprofloxacin on the microbiota occurred for the entire study period of 12 months.[106] Another study assessed the gut microbiome of three people via daily faecal sampling over a 10 month period in subjects who received two 5 day courses of ciprofloxacin separated by 6 months.[105] A notable change in the composition of the microbiota was observed in as little as 3 days after the start of the treatment course. Due to the aggressive faecal sampling, intersubject variability was captured and noted to be considerable. Importantly, up to 50% of the organisms that made up the gut microbiota prior to exposure were eliminated by the ciprofloxacin exposure. Despite having a stable microbiota composition by the eighth month of the study, each subject's microbiota make-up was altered compared with the composition noted prior to any ciprofloxacin exposure. The authors also noted that this stable change is likely due to multiple disturbances, as one subject did have a complete recovery after the first treatment course.

Conclusions

The disruptions of the microbiome from early development are known to have lasting effects far into adulthood and can even shape the person's subsequent clinical course. The molecular data describing the faecal microbiota shifts suggest that, other than the changes occurring after birth and in inflammatory manifestations of gastrointestinal diseases such as Crohn's disease, the significant change observed with antimicrobial exposures was the only other situation where these discernable microbiota alterations are evident.[107,108] Given that the microbiome and sepsis are closely intertwined, future research should expand our knowledge regarding the importance of a homeostatic microbiome and its therapeutic potential.

Although life-saving, antimicrobial interventions have significant effects on gut microbiota that are accepted in the context of the acute phase of treating a potentially fatal infection. Many studies, mostly using culture-based methods, have been done to help better understand the effects of specific antimicrobials on the faecal microbiota. However, the clinical translation of these studies is more challenging. Patients often receive multiple cocktails of therapies, are in an inflammatory state, and have confounding issues that make it difficult to delineate a direct association. Molecular-based methods may provide another level of granularity that is missed by culture-based methods. As a part of judicious stewardship, rapid technologies that identify organisms and their predicted response to antibiotic treatment (many of which do so directly from a specimen) can facilitate stewardship efforts. While it is not desirable to halt early intervention of antimicrobial therapy for patients with signs and symptoms of infection, efforts to support earlier targeted therapy should be incorporated to avoid unnecessary exposure to these agents, as many of these agents disrupt the microbiome in as little as a single dose and up to several weeks may be needed for recovery. Much like cancer chemotherapy, targeted antimicrobial therapy should be optimized to attain a balance between efficacy and toxicity.

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