Annals of Medicine

ISSN: 0785-3890 (Print) 1365-2060 (Online) Journal homepage: www.tandfonline.com/journals/iann20

Probiotic approach to prevent antibiotic resistance

Arthur C. Ouwehand, Sofia Forssten, Ashley A. Hibberd, Anna Lyra & Buffy Stahl

To cite this article: Arthur C. Ouwehand, Sofia Forssten, Ashley A. Hibberd, Anna Lyra & Buffy Stahl (2016) Probiotic approach to prevent antibiotic resistance, Annals of Medicine, 48:4, 246-255, DOI: 10.3109/07853890.2016.1161232

To link to this article: https://doi.org/10.3109/07853890.2016.1161232

ANNALS OF MEDICINE, 2016
VOL. 48, NO. 4, 246–255
http://dx.doi.org/10.3109/07853890.2016.1161232

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

Probiotic approach to prevent antibiotic resistance

Arthur C. Ouwehand[a], Sofia Forssten[a], Ashley A. Hibberd[b], Anna Lyra[a] and Buffy Stahl[c]

[a]Active Nutrition, DuPont Nutrition and Health, Kantvik, Finland; [b]DuPont Health and Nutrition, Saint Louis, MO, USA; [c]DuPont Health and Nutrition, Madison, WI, USA

ABSTRACT

Probiotics are live microorganisms, mainly belonging to the genera *Lactobacillus* and *Bifidobacterium*, although also strain of other species are commercialized, that have a beneficial effect on the host. From the perspective of antibiotic use, probiotics have been observed to reduce the risk of certain infectious disease such as certain types of diarrhea and respiratory tract infection. This may be accompanied with a reduced need of antibiotics for secondary infections. Antibiotics tend to be effective against most common diseases, but increasingly resistance is being observed among pathogens. Probiotics are specifically selected to not contribute to the spread of antibiotic resistance and not carry transferable antibiotic resistance. Concomitant use of probiotics with antibiotics has been observed to reduce the incidence, duration and/or severity of antibiotic-associated diarrhea. This contributes to better adherence to the antibiotic prescription and thereby reduces the evolution of resistance. To what extent probiotics directly reduce the spread of antibiotic resistance is still much under investigation; but maintaining a balanced microbiota during antibiotic use may certainly provide opportunities for reducing the spread of resistances.

➤ KEY MESSAGES

- Probiotics may reduce the risk for certain infectious diseases and thereby reduce the need for antibiotics.
- Probiotics may reduce the risk for antibiotic-associated diarrhea
- Probiotics do not contribute to the spread of antibiotic resistance and may even reduce it.

ARTICLE HISTORY
Received 1 February 2016
Revised 20 February 2016
Accepted 28 February 2016
Published online 25 March 2016

KEYWORDS
Antibiotic-associated diarrhea; antibiotic resistance; *Bifidobacterium*; *Enterococcus*; *Lactobacillus*; probiotics

Introduction

Antibiotics are among the most prominent advances in medicine and have provided great benefits in the treatment and control of infectious diseases. However, their wide use and especially their inappropriate use have led to the increased spread of antibiotic resistance (1). This has raised concern about the continued efficacy of antibiotics in human medicine. To counteract the spread of antibiotic resistance, first and foremost the inappropriate use of antibiotics should be reduced; both in human and veterinary applications. In addition, also alternative approaches should be considered; here probiotics could play a role. In human and veterinary medicine probiotics have been documented to reduce risk for infectious diseases (2,3); thereby potentially reducing the need for antibiotics. Furthermore, probiotics have been documented to reduce the risk for antibiotic-associated diarrhea (AAD) (4); a common side effect of antibiotic use. One of the mechanisms by which probiotics are thought to achieve this effect is by stabilization of the intestinal microbiota. This may also be a mechanism by which probiotics might influence the spread of antibiotic resistance.

Antibiotics

Antibiotics are among the most important drugs currently available to modern medicine and have enabled the treatment of otherwise deadly infectious diseases. For antibiotics to be able to provide these benefits also in the future requires a reduction in the acquisition and spread of antibiotic resistance. This starts with the intestinal microbiota; often an unintended victim of antibiotics. Antimicrobials are medicinal products that kill or stop the growth of living microorganisms; antibiotics are active against bacterial infections (European Centre for Disease Prevention and Control). By their antimicrobial nature, antibiotics may affect

CONTACT Arthur C. Ouwehand arthur.ouwehand@dupont.com Active Nutrition, DuPont Nutrition and Health, Sokeritehtaantie 20, 02460 Kantvik, Finland

more organisms then just the etiological agents of the infection for which they are used. This can lead to disturbances in particular in the gastrointestinal tract, where the majority of the body's endogenous microbes reside.

Side effects of antibiotics on intestinal microbiota

Efforts to understand the targeted effects of antibiotics on single microbes have been focused primarily on pathogens since the widespread usage of antibiotics arose in the 1940s. However, in the last decade interest in the broader ecological effects of antibiotic therapy on the host microbiota has increased (5–10). Rapid advances in high-throughput sequencing technologies have led to a realization that the resident human microbiota and its corresponding genes, or microbiome, play an integral role in human health (11,12). DNA sequencing based methods that utilize a phylogenetic biomarker such as the 16S rRNA gene are used to discern microbiota composition at the community level, while metagenomics (sequencing all microbial genetic material in a sample) and metabolomics (mass-spectrometry-based analysis of metabolites in a sample) have revealed the functional potential of our microbiome. These new technologies are beginning to uncover the complexity and importance of balanced host-microbiota interactions.

Several detrimental health conditions such as irritable bowel disease (13), AAD (14), metabolic syndrome (15) and obesity (16) have all been linked to a disturbed microbiome. The ecological consequences of antibiotics on our microbiota are primarily effects on diversity, composition and resilience of microbial communities (5,7,8). The extent of microbiome disruption caused by antibiotic treatment, both long and short term, varies both by the class of antibiotic as well as by the individual (7–9). This disruption typically occurs rapidly and individuals often begin returning to a normal state shortly after completion of the antibiotic course (5,9); however, long-term effects of antibiotics on the abundance of specific gut bacteria have been reported as long as 4-year post-treatment (17).

A recent study on the effects of antibiotic therapy on the oral (saliva) and fecal microbiota were investigated in 66 individuals taking either a placebo or one of four antibiotics: clindamycin, ciprofloxacin, amoxicillin and minocycline. The oral microbiota was more resilient to antibiotic perturbation than the fecal microbiota and recovered from exposure within 1 month. Clindamycin and ciprofloxacin had the most pronounced effects on microbial composition and diversity, with lasting effects for several months and up to 1 year, respectively. In addition, antibiotic exposure resulted in a reduction of health-associated butyrate producing bacteria and an increased abundance of antibiotic resistance genes (10). Other studies of ciprofloxacin treatment have also shown a rapid loss of microbial diversity and alteration of fecal microbial profiles (5,9). In a long-term study of repeated ciprofloxacin treatment, the recovery from disturbance was often incomplete, resulting in a microbiota that was altered from its original state (9). Infants may be particularly susceptible to long-term impacts of gut community perturbation by antibiotics due to frequent repeated antibiotic courses in early life and the immature and developing state of their microbiome (18,19).

The etiology of AAD is a classic example of gut dysbiosis manifesting as a clinical disease and it is well known that antibiotic usage increases the risk for both hospital and community acquired AAD (20,21). Opportunistic pathogens such as *Clostridium difficile*, the most common causative agent of AAD, are thought to flourish when the gut microbiota is disrupted by antibiotics (14,22,23). Probiotic and fecal microbiota transfer therapies seek to restore lost gut microbial diversity and provide colonization resistance against the proliferation of AAD-causing organisms. Specific strains of probiotic bacteria have been shown to alleviate the symptoms and incidence of AAD (more about probiotics and their effect on AAD, below in the section "Probiotics"). In a triple-blind, randomized, placebo-controlled clinical study, individuals ($n = 503$) requiring antibiotic therapy while hospitalized were given either a placebo, low (4.17×10^9 CFU) or high (1.71×10^{10} CFU) dose probiotic in conjunction with an antibiotic (penicillin, cephalosporin, or clindamycin). There was a dose–response effect on the reduction of AAD incidence and the severity of clinical AAD symptoms with probiotic supplementation (24). Additional observations on the fecal microbiota composition were made in some individuals from this study ($n = 122$) by sequencing the 16S rRNA gene marker at several time points including baseline (pre-treatment) onset of AAD, recovery and up to 4-week post-antibiotic treatment. Despite variability in microbiota profiles among the individuals due to the breadth of age, gender, antibiotic treatment, and type of illness, the onset of AAD rapidly induced a shift in the composition of the fecal microbiota (Figure 1). When AAD occurred, the microbiota composition was altered to a state that differed distinctly both from the baseline and recovery (post-antibiotic treatment) periods and from antibiotic treated non-diarrheal individuals. The gut microbiota was restored to the baseline after recovery from the diarrhea event (within 4 weeks) in most, but not all

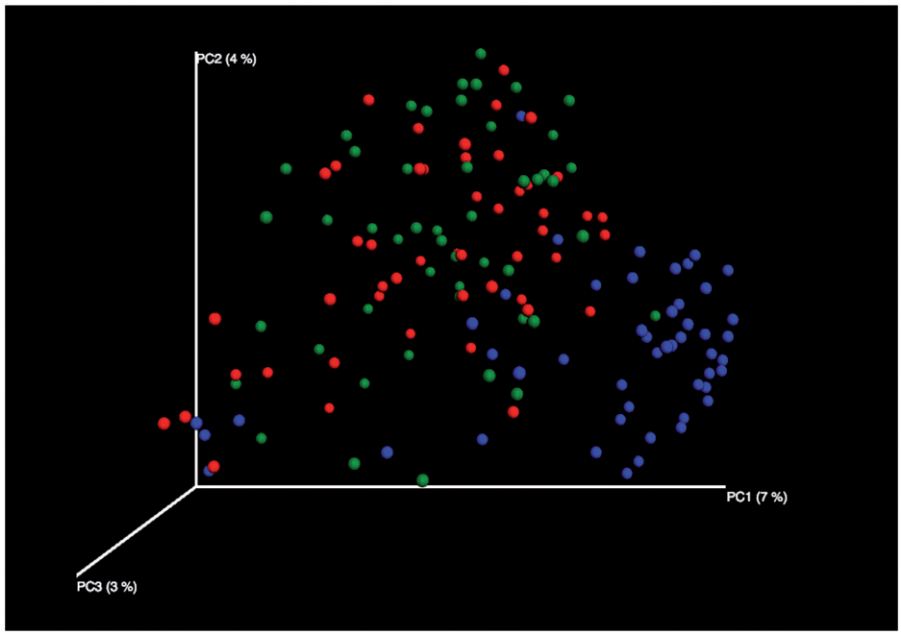

Figure 1. Fecal microbiota composition from individuals that developed antibiotic associated diarrhea (AAD). The microbiota composition was evaluated at baseline (pre-antibiotic treatment; red circles), the onset of AAD symptoms (blue circles) and 4-week post-antibiotic intervention (green circles). A distinct shift in the fecal microbiota occurred at the diarrhea event, followed by a recovery to the baseline microbiota composition in most individuals. Principal coordinates analysis (PCoA) was produced using the unweighted UniFrac metric within the QIIME v1.9.1 software package (94) on sequencing data generated using the 16S rRNA gene marker for microbiota composition.

individuals. Some individuals reached a new altered state that differed from their baseline and AAD occurrence at 4-week post-treatment; however, longer-term effects were not assessed (Hibberd AA, Forssten SD, Mao Y and Ouwehand AC, unpublished observations, 2015). These results follow trends from other antibiotic microbiome studies, where time to recovery from gut perturbation by antibiotics varies by individual and antibiotic. Though the effects of antibiotic treatment on the microbiota are often acutely drastic, many individuals complete short courses of antibiotics without immediate or outward symptoms, and the underlying long-term consequences of gut microbiota alteration due to antibiotic therapy have yet to be fully investigated.

Antibiotic resistance

Antibiotics interfere with specific molecular targets in the microbial cell. The targets may be modified or absent in different bacteria; rendering them resistant; this is intrinsic resistance and is common to most or all of the strains within a species (25). However, resistance can also be acquired (extrinsic); here different mechanisms are possible.

A mutation in a gene coding for a target of antibiotics, may make an otherwise sensitive microbe resistant to antibiotics (Figure 2). Similarly, an existing transport system may mutate and become able to expel e.g. intracellular antibiotics; making the bacterium less sensitive or even resistant (Figure 2). These forms of acquired resistance are usually present in only one or a very limited number of strains within a species. This form of resistance is also not considered transferable; except of course to daughter cells.

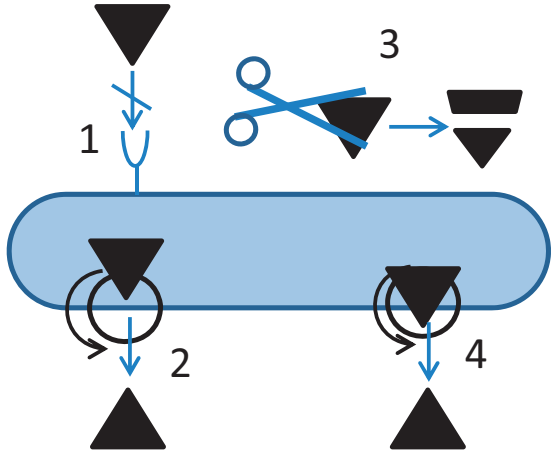

Figure 2. Schematic representation of antibiotic resistance in a bacterium: (1) target is missing or mutated and not appropriate for the antibiotic, (2) an existing exporter may also be able to expel an antibiotic, (3) a specific enzyme may inactivate the antibiotics and (4) a specific transporter may expel the antibiotic from the cell. The black triangle (▲) represents the antibiotic.

Resistance to antibiotics can also be provided by specific genes coding for mechanisms that either inactivate antibiotics; of which β-lactamases are probably the most commonly known example, or otherwise disable antibiotics (Figure 2). For intracellular active antibiotics specific export mechanisms may exist to expel the antibiotic (Figure 2). These forms of antibiotic resistance may be transferable if the genes coding for these resistances are on or near transferable elements; such as e.g. plasmids or transposons. This transferable antibiotic resistance is of greatest concern as it can be transferred to potential pathogenic bacteria making their infections potentially untreatable. Therefore it is of clinical importance to counteract this type of resistance.

Probiotics

Definition

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (26,27). Lactobacilli and bifidobacteria are the most common groups of probiotics, other genera are also been commercialized as probiotics; such as *Escherichia coli*, *Saccharomyces cerevisiae* var. boulardii and *Bacillus coagulans*. Furthermore, "new" species of beneficial microbes are identified and are being investigated for their potential as probiotics; *Akkermansia muciniphila*, *Eubacterium halii* and *Faecalibacterium prausnitzii*. Safety is an essential trait of any marketed probiotic strain, of which many have regulatory approval of safety on species level due to long history of consumption (28–30). Probiotics' health benefits may manifest themselves in several ways; e.g. through digestive, immune, metabolic and even mental health of the host. The general consensus is that health benefits are strain specific and cannot be extrapolated to other strains; not even of the same species (31). However, some properties may be common for different strains, due to similarities in metabolism of ecological functionality (26,32).

Probiotics' antibiotic resistance profiles

Antimicrobial resistance in beneficial microbes has been a focus of researchers due to the concern for increasing the risk of transfer of drug resistance(s) from microbial food products to the gut bacterial population (33–36). Regulatory agencies and industry associations have begun to address the concerns and provide guidance to assess risk of transfer from probiotics (37–39). Resistance in probiotics can be mediated by many different mechanisms that range from unknown and non-specific to fully understood and well-studied (34,38,40). The focus of the safety concern in probiotic strains has been on acquired genes that could be transferred via conjugative plasmids, transposases, and prophage/bacteriophage elements (33,41,42). Several reports have measured resistance of groups of strains of the same species to determine intrinsic microbial break point (MBP) values for comparison of antibiograms, although some probiotic species have been more thoroughly surveyed than others (41,43–47). The antibiotics relevant in human and veterinary medicine are measured in triplicate using broth microdilution methods using ISO standard; ampicillin, gentamicin, kanamycin, erythromycin, clindamycin, tetracycline, vancomycin, streptomycin, and chloramphenicol (48). The use of alternative methods or collection from diverse geographic regions can cause some variation and therefore researchers' expanding knowledge on the antibacterial spectrum of probiotic species should take note (42,47,49). Resistance to MLS antibiotics (erythromycin, clindamycin) are widely distributed in *Lactobacillus* and other organisms but some probiotic species have yet to be studied (40,44,45,48,50,51). Vancomycin resistance has been reported as intrinsic in *Lactobacillus* species and is thought to derive from point mutations rather than gene transfer (47,52). While tetracycline resistance in some lactobacilli is intrinsically high, phenotypically, it remains relatively low in other probiotic species of the genus *Lactobacillus*. *Bifidobacterium* species have been reported to ubiquitously carry a *tetW* gene, the presence of the conserved gene seems to have little to do with whether a strain is above or below the MBP (53). The β-lactams (ampicillin) resistance in LAB and *Bifidobacteria* is atypical (41,42). Intrinsic resistance to aminoglycosides (kanamycin, gentamycin, streptomycin) has been observed in several species of *Lactobacillus* (35,54). Chloramphenicol resistance in lactobacilli and bifidobacteria due to acquired *cat* resistance genes is mediated by plasmids encoding the resistance; however, point mutations in housekeeping genes have also been reported (51).

Antibiotic-associated diarrhea risk reduction

Antimicrobial treatment has a substantial effect on the composition and functional characteristics of intestinal microbiota (5,55) potentially elevating risk of AAD and *C. difficile* infection (CDI; also referred to as *C. difficile*-associated diarrhea, CDAD and *C. difficile* disease, CDD). *Clostridium difficile* is the most commonly identified pathogen in AAD. However, also other organisms have

been implicated with AAD; *Clostridium perfringens*, *Staphylococcus aureus*, *Candida* spp. and *Klebsiella* spp (56). Most notably though, is that in many cases no pathogen can be identified and the etiology may thus be viral, protozoal or related to changes in microbial composition and/or activity, such as reduced microbial metabolism of carbohydrates and primary bile acids, resulting in osmotic or secretory forms of diarrhea (57).

AAD is relatively common with a prevalence varying 5–35% depending on the type of antibiotic and length of treatment, age, concomitant diseases and hospitalization (20,21). AAD can be defined as three or more loose stools per day for at least two consecutive days (58), with no specific causative agent necessarily detected (20). CDI has a rising incidence among the hospitalized US adults; locally reaching even 15% with a 20–30% recurrence and a 9% mortality rate (25,59). In addition to reducing patients' quality of life due to AAD and/or CDI, CDI alone results in an estimated $4.8 billion of additional annual costs in the US for acute health care (59). Therefore, means to reduce the risk of AAD, when antibiotic use is mandatory, are essential.

Co-administration of probiotics during antibiotic treatment may enhance the resilience of the microbiota to antimicrobial-induced disturbances (60,61). Probiotics may reduce AAD and CDI risk also through competitive exclusion, production of bacteriocins, gut barrier reinforcement, enhancement of immune functions and balancing of intestinal transit (26,60,61). In a recent meta-analyses, the co-administration of different probiotic strains was found to be efficient in reducing the risk of AAD (62–64). McFarland et al. (62) compared 25 randomized controlled trials (RCT) with 2810 AAD patients enrolled receiving different antibiotic and probiotic treatments for varying lengths of time. Moreover, between the trials the subjects' age varied from infants to elderly with both hospitalized in-patients and out-patients included adding to heterogeneity of the data. Nevertheless, probiotics were concluded to reduce AAD risk significantly (RR 0.43, 95% CI 0.41–0.85, $p < 0.001$) with *Lactobacillus rhamnosus* GG, *S. cerevisiae* var. boulardii and multiple strain probiotic blends favored over other single strain probiotics. Likewise, a subsequent meta-analysis of *S. cerevisiae* var. boulardii supplemented RCTs (21 RCTs with 4780 participants enrolled) observed a significant level of AAD risk-reduction (RR 0.47, 95% CI 0.38–0.57, number needed to treat 10, CI 9–13) (63). The AAD risk was reduced for both adults and in children receiving antibiotics for either infection or *Helicobacter pylori* eradication. Pattani et al. (64) evaluated AAD risk reduction with probiotics among 2296 adult hospitalized patients assessed in 15 RCTs. The participants received different probiotic supplements co-administered with antibiotic treatment other than *H. pylori* eradication resulting in significant reduction of the risk for AAD for the combined trials (RR 0.61, 95% CI 0.47–0.79, NNT 11, 95% CI 8–20). Although the aforementioned meta-analyses include RCTs with differing trial settings and quality concerns, they suggest that co-administration of probiotic blends, or certain specific probiotic strains with more RCTs available, reduce the risk for AAD.

Probiotics and antibiotic resistance spread

Probiotic bacteria have many beneficial properties to control ability of pathogenic bacteria. These properties include improving intestinal barrier function, competitive exclusion e.g. by reducing adherence to cells, co-aggregation, as well as production of organic acids which antagonize pathogenic bacteria. Many probiotics produce antimicrobial compounds such as e.g., short-chain fatty acids, hydrogen peroxide, nitric oxide, and bacteriocins, and these may enhance their ability to compete against other gastrointestinal micro-organisms and could potentially inhibit pathogenic bacteria (65–68). Additionally, the production of antimicrobials is often regarded *a priori* in the context of bacterial fitness but also in terms of probiotic efficacy, since production of bacteriocin (69) has been an important criterion in the selection of a probiotic strain.

It has been shown that *Lactobacillus* strains play an effective role in the protection of the host against urinary tract infections (UTI) (70). For example *Lactobacillus rhamnosus* GR1 has been reported to efficiently bind to epithelial cells, especially in the vaginal tract (71). Furthermore, antagonistic activity of such bacteria may inhibit the binding of enteric and urinary tract bacterial pathogens (72).

The major problem with the use of antibiotics is that bacteria are able to evolve and can acquire resistance against antibiotics via several biochemical aspects; as described earlier. It has been reported that lactic acid produced by lactobacilli strains can increase the susceptibility of Gram-negative bacteria to antimicrobial agents (73). However, Naderi et al. (74) were not able to show a differences in Minimal Inhibitory Concentration (MICs) values for the antibiotic agents tested (ampicillin, clavulanic acid and cotrimoxazole) for the *E. coli* isolates before or after treatment with lactobacilli supernatants. Thus, despite earlier observations on the effect of lactic acid, it appears that lactobacilli supernatants may not able to change the antibiotic resistance patterns of the *E. coli* strains. The sensitivity of *E. coli* may be strain dependent; since

E. coli strains were isolated from urine, they may have fecal origin, thus the resistance genes isolated might be carried on plasmids inside the bacteria. For some strains plasmid deletion was detected and it was observed that abnormal colony size is due to a decrease in the number of plasmid copies not their complete deletion (74). In a study with elderly carriers of quinolone-resistant *E. coli*, probiotic *E. coli* Nissle 1917 was evaluated for excluding the resistant *E. coli*, hypothesizing the probiotic strain might occupy the same ecological niche as the resistant one. However, no difference could be detected regarding the persistence the resistant strains in the feces during therapy as compared to the placebo (75). Positively, the probiotic strain did not acquire any resistance during the trial. Thus, whether probiotics can prevent or treat multi-drug resistant organisms colonization remains uncertain.

Risk of antibiotic resistance spread with probiotics

Colonic bacteria could act as reservoirs for resistance genes that can be acquired from ingested bacteria. Although it is reasonable to assume that gene transfer from bacteria to bacteria will occur in the gastrointestinal tract, intrinsic resistance is presumed to present a minimal potential for horizontal spread, while acquired resistance is considered to have a high potential for lateral spread (76). Members of the genera *Lactococcus* and *Lactobacillus* are most commonly given "generally regarded as safe" (GRAS) or Qualified Presumption of Safety (QPS) status, while members of the genera *Streptococcus*, *Enterococcus* and some other genera of lactic acid bacteria (LAB) contain some opportunistic pathogens. Enterococcal species are have been studied for the use as probiotics in several studies (77,78), since they have some good probiotic characteristics like resistance to gastric juice and bile salts, have been shown to be stable during storage and handling, and have been documented to produce a bacteriocin (enterocin) (79). Even though enterococci generally have low pathogenicity, they increasingly are associated with nosocomial infection, especially in immunocompromised patients. This might partly be explained by the intrinsic tolerance against several antimicrobial agents and to harsh conditions, along with their ability to acquire resistance genes from other bacteria. Due to this, enterococci have a higher probability to gain antibiotic resistance genes than others in the same niche (80). Furthermore, enterococcal species are able to carry undesirable phenotypes for probiotics like the ability to produce β-hemolysin, gelatinase (81,82) as well as aggregation substance (83). Some genes that confer resistance to cephalosporins, sulfonamides and aminoglycosides are usually intrinsic, i.e., located in the chromosomes. Nevertheless, some enterococcal species may carry extrinsic resistance to chloramphenicol, erythromycin, tetracycline and vancomycin. These genes are located on plasmids or near transposons, enabling horizontally or vertically transfer to different groups of bacteria, such as to *Streptococcus* spp., *Staphylococcus aureus* and *Bacillus subtilis*, thus hampering the antimicrobial therapy (84,85). The virulence genes could also be transferred to human endogenous strains present in the gastrointestinal tract, thereby contributing to increasing virulence factors of this genus and the endogenous strains. Therefore, the safety of the enterococcal strains used as probiotics must be guaranteed, and the use as probiotics should be carefully monitored with risk/benefit analysis (86,87).

Potential benefits of probiotics in reducing antibiotic resistance; what is known

The production of bacteriocins by probiotics could be used as a way to decrease the pathogenic bacterial populations at mucosal sites as well as for disruption of biofilms (88,89) in order to improve the function of the antibiotics (90). In addition probiotics can improve the mucosal immunity, which in turn assist in the eradication of the pathogenic organisms at the mucosal site. However, most bacteriocin activity has been documented *in vitro* (89), proof that bacteriocins are also effective *in vivo* is still limited (91) even more so in humans where double blind randomized controlled trials are still scarce (92) especially concerning *in situ* production of effective bacteriocins by probiotics.

The role of probiotics in preventing drug-resistant infections in humans has not yet been established. The Center for Disease Control and Prevention (CDC) is actively researching the subject, and although some studies have shown benefit, the data is currently not conclusive enough to issue specific recommendations. Thus, so far probiotics can be used as partial replacement or adjunct to antibiotic treatment and thereby help treating multidrug resistant UTIs.

Most of the research conducted on antibiotic resistance has been focused on pathogenic bacteria prevalent in nosocomial settings like hospital and nursing home environments. However, antibiotic resistance is a much greater problem and it is also important to study the antibiotic resistance in food animal production as well as aquaculture. Antibiotic resistance genes are often located on mobile genetic elements, thus it is important to determine how these elements move

through the entire food ecosystem. Although antibiotic growth promoters (AGPs) were banned in Europe in 2006, they are still used as AGPs in other parts of the world. In addition, in Europe, antibiotics are still widely used in veterinary medicine for the treatment of disease in production animals. We need to understand the resistance ecology, thus more targeted interventions to reduce the selection pressure on the emergence, evolution, and spread of antibiotic resistance are needed. By using probiotics in the feed and thus improve animal health, the spread of antibiotic resistance might be reduced.

Conclusions

One of the best documented benefits of probiotics is in relation to antibiotic use, with many probiotic strains having been reported to have beneficial effects. Most of these benefits are in relation to AAD, but also maintenance of the intestinal and other microbiota composition and activity. This can be expected to contribute to a reduced spread or evolution of antibiotic resistance. For the future, probiotic studies investigating the influence on antibiotic use should look for the presence or absence of relevant resistance genes to get a better understanding on the opportunities (93). The opportunities are there, so are the tools, it should therefore receive more attention since on a global perspective reducing resistance spread may be a bigger benefit then reductions in AAD.

Disclosure statement

All authors are employees of DuPont. DuPont manufactures and markets probiotics.

References

1. Sabtu N, Enoch DA, Brown NM. Antibiotic resistance: what, why, where, when and how? Br Med Bull. 2015;116:105–13.
2. King S, Glanville J, Sanders ME, Fitzgerald A, Varley D. Effectiveness of probiotics on the duration of illness in healthy children and adults who develop common acute respiratory infectious conditions: a systematic review and meta-analysis. Br J Nutr. 2014;112:41–54.
3. Applegate JA, Fischer Walker CL, Ambikapathi R, Black RE. Systematic review of probiotics for the treatment of community-acquired acute diarrhea in children. BMC Publ Health. 2013;13:S16.
4. Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JN, Shanman R, et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. JAMA. 2012;307:1959–69.
5. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. 2008;6:e280.
6. Macfarlane S. Antibiotic treatments and microbes in the gut. Environ Microbiol. 2014;16:919–24.
7. Rashid MU, Zaura E, Buijs MJ, Keijser BJ, Crielaard W, Nord CE, et al. Determining the long-term effect of antibiotic administration on the human normal intestinal microbiota using culture and pyrosequencing methods. Clin Infect Dis. 2015;60:S77–84.
8. Zaura E, Brandt BW, Teixeira de Mattos MJ, Buijs MJ, Caspers MP, Rashid MU, et al. Same exposure but two radically different responses to antibiotics: resilience of the salivary microbiome versus long-term microbial shifts in feces. mBio. 2015;6:e01693–15.
9. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci USA. 2011;108:4554–61.
10. Modi SR, Collins JJ, Relman DA. Antibiotics and the gut microbiota. J Clin Invest. 2014;124:4212–8.
11. Belizario JE, Napolitano M. Human microbiomes and their roles in dysbiosis, common diseases, and novel therapeutic approaches. Front Microbiol. 2015;6:1050.
12. Althani A, Marei HE, Hamdi WS, Nasrallah GK, El Zowalaty ME, Al Khdor S, et al. Human microbiome and its association with health and diseases. J Cell Physiol. 2015. [Epub ahead of print]. doi: 10.1002/jcp.25284.
13. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol. 2012;13:R79.
14. Young VB, Schmidt TM. Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. J Clin Microbiol. 2004;42:1203–6.
15. Cani PD, Delzenne NM. The role of the gut microbiota in energy metabolism and metabolic disease. Curr Pharm Des. 2009;15:1546–58.
16. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature. 2009;457:480–4.
17. Jakobsson HE, Jernberg C, Andersson AF, Sjolund-Karlsson M, Jansson JK, Engstrand L. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PloS One. 2010;5:e9836.
18. Nobel YR, Cox LM, Kirigin FF, Bokulich NA, Yamanishi S, Teitler I, et al. Metabolic and metagenomic outcomes from early-life pulsed antibiotic treatment. Nat Commun. 2015;6:7486.
19. Saari A, Virta LJ, Sankilampi U, Dunkel L, Saxen H. Antibiotic exposure in infancy and risk of being overweight in the first 24 months of life. Pediatrics. 2015;135:617–26.
20. Wistrom J, Norrby SR, Myhre EB, Eriksson S, Granstrom G, Lagergren L, et al. Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalized patients: a prospective study. J Antimicrob Chemother. 2001;47:43–50.

21. McFarland LV. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. Dig Dis. 1998;16:292–307.
22. Schubert AM, Rogers MA, Ring C, Mogle J, Petrosino JP, Young VB, et al. Microbiome data distinguish patients with *Clostridium difficile* infection and non-*C. difficile*-associated diarrhea from healthy controls. mBio. 2014;5:e01021-14.
23. Knecht H, Neulinger SC, Heinsen FA, Knecht C, Schilhabel A, Schmitz RA, et al. Effects of beta-lactam antibiotics and fluoroquinolones on human gut microbiota in relation to *Clostridium difficile* associated diarrhea. PloS One. 2014;9:e89417.
24. Ouwehand AC, DongLian C, Weijian X, Stewart M, Ni J, Stewart T, et al. Probiotics reduce symptoms of antibiotic use in a hospital setting: a randomized dose response study. Vaccine. 2014;32:458–63.
25. Reveles KR, Lee GC, Boyd NK, Frei CR. The rise in *Clostridium difficile* infection incidence among hospitalized adults in the United States: 2001–2010. Am J Infect Control. 2014;42:1028–32.
26. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506–14.
27. FAO/WHO. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Food and Agriculture Organization and World Health Organization [online]. 2001.
28. EFSA. Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the maintenance of the list of QPS microorganisms intentionally added to food or feed (2009 update). EFSA J. 2009;7:92.
29. Bourdichon F, Casaregola S, Farrokh C, Frisvad JC, Gerds ML, Hammes WP, et al. Food fermentations: microorganisms with technological beneficial use. Int J Food Microbiol. 2012;154:87–97.
30. Mogensen G, Salminen S, O'Brien J, Ouwehand A, Holzapfel W, Shortt C, et al. Inventory of microorganisms with a documented history of use in food, p. 10. Brussels: International Dairy Federation; 2002.
31. Fijan S. Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Publ Health. 2014;11:4745–67.
32. Bermudez-Brito M, Plaza-Diaz J, Munoz-Quezada S, Gomez-Llorente C, Gil A. Probiotic mechanisms of action. Ann Nutr Metab. 2012;61:160–74.
33. Nawaz M, Wang J, Zhou A, Ma C, Wu X, Moore JE, et al. Characterization and transfer of antibiotic resistance in lactic acid bacteria from fermented food products. Curr Microbiol. 2011;62:1081–9.
34. Wang HH, Manuzon M, Lehman M, Wan K, Luo H, Wittum TE, et al. Food commensal microbes as a potentially important avenue in transmitting antibiotic resistance genes. FEMS Microbiol Lett. 2006;254:226–31.
35. Marshall BM, Ochieng DJ, Levy SB. Commensals: underappreciated reservoir of antibiotic resistance. Microbes. 2009;4:231–8.
36. Zhang L, Kinkelaar D, Huang Y, Li Y, Li X, Wang HH. Acquired antibiotic resistance: are we born with it? Appl Environ Microbiol. 2011;77:7134–41.
37. EFSA. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA J. 2012;10:2740.
38. Gueimonde M, Florez AB, van Hoek AH, Stuer-Lauridsen B, Stroman P, de los Reyes-Gavilan CG, et al. Genetic basis of tetracycline resistance in Bifidobacterium animalis subsp. lactis. Appl Environ Microbiol. 2010;76:3364–9.
39. Hazards EPoB. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2012 update). EFSA J. 2012;10:3020.
40. Florez AB, Ladero V, Alvarez-Martin P, Ammor MS, Alvarez MA, Mayo B. Acquired macrolide resistance in the human intestinal strain Lactobacillus rhamnosus E41 associated with a transition mutation in 23S rRNA genes. Int J Antimicrob Agents. 2007;30:341–4.
41. Mathur S, Singh R. Antibiotic resistance in food lactic acid bacteria – a review. Int J Food Microbiol. 2005;105:281–95.
42. Ouoba LI, Lei V, Jensen LB. Resistance of potential probiotic lactic acid bacteria and bifidobacteria of African and European origin to antimicrobials: determination and transferability of the resistance genes to other bacteria. Int J Food Microbiol. 2008;121:217–24.
43. Danielsen M, Wind A. Susceptibility of Lactobacillus spp. to antimicrobial agents. Int J Food Microbiol. 2003;82:1–11.
44. Gueimonde M, Sanchez B, GdLR-G C, Margolles A. Antibiotic resistance in probiotic bacteria. Front Microbiol. 2013;4:202.
45. Rojo-Bezares B, Saenz Y, Poeta P, Zarazaga M, Ruiz-Larrea F, Torres C. Assessment of antibiotic susceptibility within lactic acid bacteria strains isolated from wine. Int J Food Microbial. 2006;111:234–40.
46. Masco L, Van Hoorde K, De Brandt E, Swings J, Huys G. Antimicrobial susceptibility of Bifidobacterium strains from humans, animals and probiotic products. J Antimicrob Chemother. 2006;58:85–94.
47. Klare I, Konstabel C, Werner G, Huys G, Vankerckhoven V, Kahlmeter G, et al. Antimicrobial susceptibilities of Lactobacillus, Pediococcus and Lactococcus human isolates and cultures intended for probiotic or nutritional use. J Antimicrob.Chemother. 2007;59:900–12.
48. ISO/IDF. Milk and milk products – determination of the minimal inhibitory concentration (MIC) of antibiotic applicable to bifidobacteria and non-enterococcal lactic acid bacteria (LAB). 2010 Contract No.: International Dairy Federation 223; 2010.
49. Florez AB, Danielsen M, Korhonen J, Zycka J, von Wright A, Bardowski J, et al. Antibiotic survey of Lactococcus lactis strains to six antibiotics by Etest, and establishment of new susceptibility-resistance cut-off values. J Dairy Res. 2007;74:262–8.
50. Drago L, Mattina R, Nicola L, Rodighiero V, De Vecchi E. Macrolide resistance and in vitro selection of resistance to antibiotics in Lactobacillus isolates. J Microbiol. 2011;49:651–6.

51. Roberts MC. Update on macrolide-lincosamide-streptogramin, ketolide, and oxazolidinone resistance genes. FEMS Microbiol Lett. 2008;282:147–59.
52. Swenson JM, Facklam RR, Thornsberry C. Antimicrobial susceptibility of vancomycin-resistant Leuconostoc, Pediococcus, and Lactobacillus species. Antimicrob Agents Chemother. 1990;34:543–9.
53. Aires J, Doucet-Populaire F, Butel MJ. Tetracycline resistance mediated by tet(W), tet(M), and tet(O) genes of Bifidobacterium isolates from humans. Appl Environ Microbiol. 2007;73:2751–4.
54. del Campo R, Tenorio C, Rubio C, Castillo J, Torres C, Gomez-Lus R. Aminoglycoside-modifying enzymes in high-level streptomycin and gentamicin resistant Enterococcus spp. in Spain. Int J Antimicrob Agents. 2000;15:221–6.
55. Raymond F, Ouameur AA, Deraspe M, Iqbal N, Gingras H, Dridi B, et al. The initial state of the human gut microbiome determines its reshaping by antibiotics. ISME J. 2016;10:707–20.
56. Gorkiewicz G. Nosocomial and antibiotic-associated diarrhoea caused by organisms other than *Clostridium difficile*. Int J Antimicrob Agents. 2009;33:S37–41.
57. Song HJ, Shim KN, Jung SA, Choi HJ, Lee MA, Ryu KH, et al. Antibiotic-associated diarrhea: candidate organisms other than *Clostridium difficile*. Korean J Intern Med. 2008;23:9–15.
58. Katz DA, Lynch ME, Littenberg B. Clinical prediction rules to optimize cytotoxin testing for *Clostridium difficile* in hospitalized patients with diarrhea. Am J Med. 1996;100:487–95.
59. Evans CT, Safdar N. Current trends in the epidemiology and outcomes of *Clostridium difficile* infection. Clin Infect Dis. 2015;60:S66–71.
60. Plummer SF, Garaiova I, Sarvotham T, Cottrell SL, Le Scouiller S, Weaver MA, et al. Effects of probiotics on the composition of the intestinal microbiota following antibiotic therapy. Int J Antimicrob Agents. 2005;26:69–74.
61. Engelbrektson A, Korzenik JR, Pittler A, Sanders ME, Klaenhammer TR, Leyer G, et al. Probiotics to minimize the disruption of faecal microbiota in healthy subjects undergoing antibiotic therapy. J Med Microbiol. 2009;58(Pt 5):663–70.
62. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease. Am J Gastroenterol. 2006;101:812–22.
63. Szajewska H, Kolodziej M. Systematic review with meta-analysis: *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea. Aliment Pharmacolther. 2015;42:793–801.
64. Pattani R, Palda VA, Hwang SW, Shah PS. Probiotics for the prevention of antibiotic-associated diarrhea and *Clostridium difficile* infection among hospitalized patients: systematic review and meta-analysis. Open Med. 2013;7:e56–67.
65. Atassi F, Servin AL. Individual and co-operative roles of lactic acid and hydrogen peroxide in the killing activity of enteric strain *Lactobacillus johnsonii* NCC933 and vaginal strain *Lactobacillus gasseri* KS120.1 against enteric, uropathogenic and vaginosis-associated pathogens. FEMS Microbiol Lett. 2010;304:29–38.
66. Chenoll E, Casinos B, Bataller E, Astals P, Echevarria J, Iglesias JR, et al. Novel probiotic *Bifidobacterium bifidum* CECT 7366 strain active against the pathogenic bacterium *Helicobacter pylori*. Appl Environ Microbiol. 2011;77:1335–43.
67. De Vuyst L, Leroy F. Bacteriocins from lactic acid bacteria: production, purification, and food applications. J Mol Microbiol Biotechnol. 2007;13:194–9.
68. Ennahar S, Sashihara T, Sonomoto K, Ishizaki A. Class IIa bacteriocins: biosynthesis, structure and activity. FEMS Microbiol Rev. 2000;24:85–106.
69. Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CGM. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. Proc Natl Acad Sci. 2007;104:7617–21.
70. Falagas ME, Betsi GI, Tokas T, Athanasiou S. Probiotics for prevention of recurrent urinary tract infections in women: a review of the evidence from microbiological and clinical studies. Drugs. 2006;66:1253–61.
71. Reid G. In vitro testing of *Lactobacillus acidophilus* NCFMTM as a possible probiotic for the urogenital tract. Int Dairy J. 2000;10:415–9.
72. Hutt P, Shchepetova J, Loivukene K, Kullisaar T, Mikelsaar M. Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. J Appl Microbiol. 2006;100:1324–32.
73. Alakomi HL, Paananen A, Suihko ML, Helander IM, Saarela M. Weakening effect of cell permeabilizers on Gram-negative bacteria causing biodeterioration. Appl Environ Microbiol. 2006;72:4695–703.
74. Naderi A, Kasra-Kermanshahi R, Gharavi S, Imani Fooladi AA, Abdollahpour Alitappeh M, Saffarian P. Study of antagonistic effects of Lactobacillus strains as probiotics on multi drug resistant (MDR) bacteria isolated from urinary tract infections (UTIs). Iran J Basic Med Sci. 2014;17:201–8.
75. Tannock GW, Tiong IS, Priest P, Munro K, Taylor C, Richardson A, et al. Testing probiotic strain Escherichia coli Nissle 1917 (Mutaflor) for its ability to reduce carriage of multidrug-resistant *E. coli* by elderly residents in long-term care facilities. J Med Microbiol. 2011;60:366–70.
76. Devirgiliis C, Barile S, Perozzi G. Antibiotic resistance determinants in the interplay between food and gut microbiota. Genes Nutr. 2011;6:275–84.
77. Pieniz S, Andreazza R, Anghinoni T, Camargo F, Brandelli A. Probiotic potential, antimicrobial and antioxidant activities of *Enterococcus durans* strain LAB18s. Food Control. 2014;37:251–6.
78. Sivieri K, Spinardi-Barbisan ALT, Barbisan LF, Bedani R, Pauly ND, Carlos IZ, et al. Probiotic *Enterococcus faecium* CRL 183 inhibit chemically induced colon cancer in male Wistar rats. Eur Food Res Technol. 2008;228:231–7.
79. Saarela M, Mogensen G, Fonden R, Matto J, Mattila-Sandholm T. Probiotic bacteria: safety, functional and technological properties. J Biotechnol. 2000;84:197–215.

80. Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in enterococcus. Virulence. 2012;3:421–569.
81. Belgacem ZB, Abriouel H, Omar NB, Lucas R, Martínez-Canamero M, Gálvez A, et al. Antimicrobial activity, safety aspects, and some technological properties of bacteriocinogenic *Enterococcus faecium* from artisanal Tunisian fermented meat. Food Control. 2010;21:462–70.
82. Giridhara Upadhyaya PM, Umapathy BL, Ravikumar KL. Comparative study for the presence of enterococcal virulence factors gelatinase, hemolysin and biofilm among clinical and commensal isolates of *Enterococcus faecalis*. J Lab Phys. 2010;2:100–4.
83. Sartingen S, Rozdzinski E, Muscholl-Silberhorn A, Marre R. Aggregation substance increases adherence and internalization, but not translocation, of *Enterococcus faecalis* through different intestinal epithelial cells in vitro. Infect Immun. 2000;68:6044–7.
84. Chow JW. Aminoglycoside resistance in Enterococci. Clin Infect Dis. 2000;31:586–9.
85. Donabedian SM, Thal LA, Hershberger E, Perri MB, Chow JW, Bartlett P, et al. Molecular characterization of gentamicin-resistant enterococci in the United States: evidence of spread from animals to humans through food. J Clin Microbiol. 2003;41:1109–13.
86. Franz CM, Huch M, Abriouel H, Holzapfel W, Galvez A. Enterococci as probiotics and their implications in food safety. Int J Food Microbiol. 2011;151:125–40.
87. Bhardwaj A, Kaur G, Gupta H, Vij S, Malik RK. Interspecies diversity, safety and probiotic potential of bacteriocinogenic *Enterococcus faecium* isolated from dairy food and human faeces. World J Microbiol Biotechnol. 2010;27:591–602.
88. Riboulet-Bisson E, Sturme MH, Jeffery IB, O'Donnell MM, Neville BA, Forde BM, et al. Effect of *Lactobacillus salivarius* bacteriocin Abp118 on the mouse and pig intestinal microbiota. PloS One. 2012;7:e31113.
89. Lin X, Chen X, Chen Y, Jiang W, Chen H. The effect of five probiotic lactobacilli strains on the growth and biofilm formation of Streptococcus mutans. Oral Dis. 2015;21:e128–34.
90. Vuotto C, Longo F, Donelli G. Probiotics to counteract biofilm-associated infections: promising and conflicting data. Int J Oral Sci. 2014;6:189–94.
91. Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CG. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. Proc Natl Acad Sci USA. 2007;104:7617–21.
92. Kang BS, Seo JG, Lee GS, Kim JH, Kim SY, Han YW, et al. Antimicrobial activity of enterocins from *Enterococcus faecalis* SL-5 against Propionibacterium acnes, the causative agent in acne vulgaris, and its therapeutic effect. J Microbiol. 2009;47:101–9.
93. Forssten S, Evans M, Wilson D, Ouwehand AC. Influence of a probiotic mixture on antibiotic induced microbiota disturbances. World J Gastroenterol. 2014;20:11878–85.
94. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335–6.