Antibiotic-Induced Perturbations of the Intestinal Microbiota Alter Host Susceptibility to Enteric Infection

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Intestinal microbiota comprises microbial communities that reside in the gastrointestinal tract and are critical to normal host physiology. Understanding the microbiota’s role in host response to invading pathogens will further advance our knowledge of host-microbe interactions. Salmonella enterica serovar Typhimurium was used as a model enteric pathogen to investigate the effect of intestinal microbiota perturbation on host susceptibility to infection. Antibiotics were used to perturb the intestinal microbiota. C57BL/6 mice were treated with clinically relevant doses of streptomycin and vancomycin in drinking water for 2 days, followed by oral infection with Salmonella enterica serovar Typhimurium. Alterations in microbiota composition and numbers were evaluated by fluorescent in situ hybridization, differential plating, and Sybr green staining. Antibiotics had a dose-dependent effect on intestinal microbiota composition. The chosen antibiotic regimen did not significantly alter the total numbers of intestinal bacteria but altered the microbiota composition. Greater preinfection perturbations in the microbiota resulted in increased mouse susceptibility to Salmonella serovar Typhimurium intestinal colonization, greater postinfection alterations in the microbiota, and more severe intestinal pathology. These results suggest that antibiotic treatment alters the balance of the microbial community, which predisposes the host to Salmonella serovar Typhimurium infection, demonstrating the importance of a healthy microbiota in host response to enteric pathogens.

Intestinal microbiota, or intestinal normal flora, is composed of a highly complex community of various microorganisms, among them bacteria. Members of the bacterial community contribute to many aspects of intestinal tract development and provide metabolic contributions well in excess of the human genome (14, 31, 32). A healthy microbial community is essential for the health of the host; conversely, imbalances of the microbiota have the potential to promote illness and contribute to the establishment and persistence of various diseases. Bacteria comprising the intestinal microbiota cluster within a number of phyla. Firmicutes and Cytophaga-Flavobacterium-Bacteroidetes (CFB) are the two phyla that make up over 90% of both the human and murine intestinal microbiotas (12, 24), with Proteobacteria, Actinobacteria, Verrucomicrobia, and Cyanobacteria comprising a much smaller portion. While the compositions of intestinal microbiotas at the phylum level are similar among individuals, and even between humans and mice, high numbers of different species are found within each phylum, with a lot of interindividual variations (12, 14, 24).

Many diseases have been linked to imbalances in the intestinal microbial community, including obesity (41), inflammatory bowel disease (13), colorectal cancer (46), and even atopic diseases (25, 34). For most of these associations, however, it is not clear whether the microbial imbalance is a predisposing factor that precedes the onset of the pathology or is the result of the pathological condition. Diseases such as vaginal candidiasis and Clostridium difficile colitis frequently start following a course of antibiotic therapy that disrupts the microbiota balance (11, 39), which favors the hypothesis that the imbalance precedes the onset of pathology. However, whether this conclusion can be extrapolated to all disorders in question is not known. Furthermore, little is known about the effect of perturbations in the intestinal microbiota on host susceptibility to invading pathogens. It has recently been demonstrated that enteric pathogens interact extensively with the intestinal microbiota (4, 21, 27, 40), thereby prompting the hypothesis that different microbiota compositions, as a result of perturbations in the microbial community, affect the outcome of enteric infections.

To investigate this hypothesis, we disrupted the murine intestinal microbial community with various doses of streptomycin and vancomycin and characterized the achieved perturbations. The effect of antibiotics on intestinal microbiota has been poorly evaluated beyond studies of induction of resistance (26, 35, 47). Additionally, most previous studies used culture techniques to evaluate the effect of tested antibiotics on microbiota (17, 36, 45), severely limiting the scope of assessment. Our evaluation involved culture-independent techniques, providing a novel glimpse at the extent of antibiotic-induced changes in the intestinal microbiota. Streptomycin was chosen as a nonspecific broad-spectrum antibiotic and vancomycin as an antibiotic with a gram-positive spectrum of activity. Both antibiotics are used in clinical practice for a wide variety of infections (Table 1).
After the antibiotic treatment, mice were infected with *Salmonella enterica* serovar Typhimurium to investigate the effects of the preinfection perturbation on the outcome of infection. *Salmonella* serovar Typhimurium is the causative agent of enteric salmonellosis in humans and is often used as a model of typhoid fever in mice. It was chosen as the model pathogen since the normal intestinal microbiota of mice provides a barrier to *Salmonella* serovar Typhimurium-induced intestinal disease (37), making the effects of any perturbation in the microbial community more obvious and easier to evaluate. The doses of antibiotics used in this study were considerably lower than those used in the conventional streptomycin *Salmonella* serovar Typhimurium infection model (5, 40) and did not significantly reduce the total number of intestinal microbes, providing a better insight into their role in *Salmonella* serovar Typhimurium-induced disease.

We found that the degree of antibiotic-induced microbial perturbation prior to infection was associated with the ability of *Salmonella* serovar Typhimurium to colonize the mouse intestinal tract, further perturb the intestinal microbiota, and induce intestinal pathology. A change in the composition alone of the intestinal microbiota (in the absence of significant changes in total numbers of intestinal microbes) prior to *Salmonella* serovar Typhimurium infection was sufficient to make mice more susceptible to *Salmonella* serovar Typhimurium, indicating that different subsets of the microbiota may play protective roles or enhance susceptibility to enteric infections.

### MATERIALS AND METHODS

**Mice.** C57BL/6 female mice (Jackson Laboratory, Bar Harbor, ME) were housed in the animal facility at the University of British Columbia (UBC) in accordance with guidelines of the UBC Animal Care Committee and the Canadian Council on the Use of Laboratory Animals and infected at 4.5 to 5.5 weeks of age. Mice were fed a standard sterile chow diet (Laboratory Rodent Diet 5001, Purina Mills, St. Louis, Missouri) ad libitum throughout experiments. Control mice received sterilized but not acidified water throughout the experiment.

**Bacterial strains.** *Salmonella* serovar Typhimurium strain SL1344 (16) was grown at 37°C with shaking (200 rpm) overnight in Luria-Bertani (LB) broth supplemented with 100 µg/ml streptomycin. The strain is resistant to vancomycin and streptomycin.

**Mouse infection.** Mice were treated with streptomycin (Sigma) at 150, 300, and 450 mg/liter and vancomycin (Sigma) at 50 and 100 mg/liter in drinking water for 2 days. As mice drink, on average, 3 ml of liquid per day (6), the average consumed dose of antibiotics was calculated (Table 1). Control mice were given drinking water without antibiotics. After 2 days, the antibiotics were withdrawn and mice were infected with 2.7 × 10⁶ *Salmonella* serovar Typhimurium CFU/mouse by oral gavage. Uninfected control mice were given 100 µl of sterile LB broth. At 3 days postinfection, the mice were euthanized by CO₂ asphyxiation and tissues were harvested aseptically for further evaluation.

**Tissue collection and bacterial enumeration.** Cecal and colons were collected in 1 ml of sterile phosphate-buffered saline on ice and homogenized with an MM 301 mixer mill (Retsch, Newtown, PA). Serial dilutions of the homogenates were plated on LB or xylene-lysine-deoxycholate (Oxoid) agar plates containing 100 µg/ml streptomycin to enumerate *Salmonella* serovar Typhimurium colonization. Serial dilutions were plated on LB, MacConkey (Oxoid), kanamycin-esculin-azide (EMD Chemicals), and Rogosa (Oxoid) agar plates to enumerate colonization by culturable aerobes, *Enterobacteriaceae*, enterococci group D streptococci, and lactobacilli, respectively. All plates except Rogosa were incubated aerobically at 37°C overnight; Rogosa plates were incubated anaerobically with GasPack Plus anaerobic system envelopes (BD) for 2 days.

**Histopathology.** Cecal tips were fixed in 10% neutral buffered formalin overnight and then placed into 75% ethanol. Fixed tissues were embedded in paraffin and cut into 5-µm sections. Tissues were stained with hematoxylin and eosin (H&E), using standard techniques by Wax-it Histology Services (Vancouver, BC, Canada) and the UBC Histology Laboratory. Pathological scores were assigned as previously described (9). Pathology images were taken using a Zeiss Axioskop 2 microscope.

**Fluorescence microscopy.** A 1:10 dilution of each organ homogenate was stored in 3.7% formalin at 4°C until use. Two to 40 µl of the samples was stained with 0.25 µl Sybr green (Invitrogen) as previously described (27) and viewed with an Olympus IX81 microscope. Three fields were randomly chosen; the numbers of cells were counted and averaged. The counts were made in a microscope field of a known diameter and corrected to the volume of sample used. For fluorescent in situ hybridization (FISH), as previously described (27), 5 to 100 µl of the samples (with 100 µl of sample being the limit of detection) was hybridized to 250 ng of the general *EUB383* probe (5'-GCT GCC TCC CGT AGG AGT-3') (2) fluorescently labeled with Texas Red and 250 ng of either *CFB286* probe (5'-TCC TCT CAG AAC CCC TAC-3') (43) or *GAM42a* probe (5'-GCC TTC CCA CAT CGT TT-3') (28) labeled with fluorescein and viewed and counted as described above. The percent compositions of the CFb and *Gammaproteobacteria* phyla were determined by dividing the numbers obtained for these phyla (CFB286 and GAM42a probes, respectively) by the numbers obtained for all eubacteria (*EURO383* probe). The percent composition of *Firmicutes* and “other” bacteria was determined by subtracting the results obtained for CFb and *Gammaproteobacteria* from 100%.

**ELISAs.** Cecum homogenates were centrifuged twice for 10 min at 13,000 g, and the supernatants were collected and stored at −80°C. The levels of tumor necrosis factor alpha (TNF-α), monocyte chemotactic protein 1 (MCP-1), interleukin-6 (BD Biosciences), and keratinocyte chemoattractant protein-1 (R&D Systems) were determined by enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer’s instructions. Cytokine levels were normalized to the total protein levels in the samples, as determined by the Bradford assay (7a).

**Statistical analysis.** One-way analysis of variance (ANOVA) with Bonferroni’s posttest or Kruskal-Wallis with Dunn’s posttest was performed using a 95% confidence interval. All analyses were performed using GraphPad Prism version 4.0. Differences were considered to be significant at P values of <0.05.

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**TABLE 1. Antibiotics used in the study**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dose (mg/liter)</th>
<th>Mouse dose (mg/kg/day)</th>
<th>Dose received over 2 days (mg)</th>
<th>Clinical dosage and administration</th>
<th>Clinical uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>150</td>
<td>30</td>
<td>0.9</td>
<td>15 mg/kg (up to 2 g); 3 times per wk to daily for 2 wk–3 mo, depending on indication; i.m. or i.v.; 2–4 g over 2 days for avg 70-kg adult</td>
<td>Tuberculosis, brucellosis, plague, endocarditis, tularemia; given in combination therapy</td>
</tr>
</tbody>
</table>
|              | 300            | 60                     | 1.8                           | 20–40 mg/kg (up to 1 g) daily; 10–14 days and up to 5 mo, depending on indication; i.m. or i.v.; 0.8–1.6 g over 2 days for avg 20-kg child | [
|              | 450            | 90                     | 2.7                           |                                     |               |
| Vancomycin   | 50             | 10                     | 0.3                           | 0.5–2 g daily for 7–10 days; p.o. or i.v.; 1–4 g over 2 days for avg adult | Staphylococcal and streptococcal infections, endocarditis, C. difficile–associated diarrhea and colitis; given alone or in combination therapy |
|              | 100            | 20                     | 0.6                           | 40 mg/kg (up to 2 g) daily for 7–10 days; p.o. or i.v.; 1.6 g over 2 days for avg 20-kg child |               |

* a i.m., intramuscular; i.v., intravenous; p.o., oral.

b See reference 30.
Vancomycin withdrawal, mice were infected with 2.7 antibiotic pretreatment. Mice were treated with the specified antibiotics in drinking water for 2 days. Doses are in mg/liter. After antibiotic withdrawal, mice were infected with 2.7 antibiotic pretreatment. Mice were treated with the specified antibiotics in drinking water for 2 days. Doses are in mg/liter. (B) Microbiota composition in colons of Salmonella serovar Typhimurium-infected mice with and without antibiotic treatment. Mice were treated with the specified antibiotics in drinking water for 2 days. Doses are in mg/liter. After antibiotic withdrawal, mice were infected with 2.7 × 10⁸ CFU of Salmonella serovar Typhimurium for 3 days. Groups marked with an asterisk have a proportion of Gammaproteobacteria significantly different from that of the control (C) group (P < 0.05). (C) Microbiota composition in ceca of Salmonella serovar Typhimurium-infected mice with and without antibiotic pretreatment. Mice were treated with the specified antibiotics in drinking water for 2 days. Doses are in mg/liter. After antibiotic withdrawal, mice were infected with 2.7 × 10⁸ CFU of Salmonella serovar Typhimurium for 3 days. Groups marked with an asterisk have a proportion of Gammaproteobacteria significantly different from that of the C group (P < 0.05).

**RESULTS**

Antibiotic treatment modifies the microbiota composition in an antibiotic- and dose-dependent manner but does not significantly change the total numbers of intestinal microbiota.

To evaluate the effects of streptomycin and vancomycin on the intestinal microbiota of mice, we employed FISH and differential plating methods. FISH provides a good overview of the distribution of the major phyla in the intestinal microbiota and was previously shown to correlate well with the results of the more laborious and expensive sequencing method (27). Differential plating provides an evaluation of a selection of bacteria at a finer genus level. Colonic microbiota of antibiotic-treated uninfected mice was evaluated. Mice were treated with increasing doses of streptomycin and vancomycin, administered in drinking water for 2 days. The dosage was either within the range of or lower than what is used in clinical practice for several indications (Table 1).

As demonstrated in Fig. 1A, the treatment of mice with increasing doses of streptomycin gradually increased the proportion of CFB bacteria in their colonic microbiotas in a dose-dependent manner. At the genus level, the numbers of lactobacilli and enterococci/group D streptococci significantly decreased with streptomycin treatment (Fig. 2A), consistent with a gradual decrease in the proportion of Firmicutes and “other” bacteria as evidenced by FISH evaluation.

The effect of vancomycin on the microbiota was strikingly different from the effect of streptomycin (Fig. 1A). The low vancomycin dose caused a dramatic reduction in the proportion of CFB bacteria and a small increase in the proportion of Gammaproteobacteria. With a higher vancomycin dose, the proportion of the CFB bacteria was still reduced, and that of the Gammaproteobacteria increased to nearly 50% of the microbiota. At the genus level, vancomycin treatment adversely affected both lactobacilli and enterococci/group D streptococci and promoted the overgrowth of Enterobacteriaceae and culturable aerobic bacteria in a dose-dependent manner (Fig. 2A). Overall, the evaluation of bacteria at the genus level for all groups of mice showed variation between mice, consistent with the fact that individuals display variation in their microbiota composition at the species level (12, 14, 24).
the selected antibiotics on lactobacilli, although statistically significant, was not as dramatic as the effect on other bacterial groups and potentially not as significant biologically. Treatment with antibiotics alone did not significantly change the total numbers of microbiota (as determined by Sybr green staining), although treatment with vancomycin had a greater effect on the total numbers of microbiota than treatment with streptomycin (Fig. 3A). However, even the largest reduction in numbers of microbiota observed was less than a log different from numbers observed for the control group.

**Preinfection alterations in the microbiota promote further infection-induced modifications.** Next, the intestinal micro-
biota of mice following infection with Salmonella serovar Typhimurium was examined, using the methods described above. When untreated and antibiotic-treated mice were infected with Salmonella serovar Typhimurium, a striking feature was an increase in the proportion of Gammaproteobacteria in all infected groups (Fig. 1B and C). Examination of both the colonic and cecal microbiotas yielded very similar results at both the phylum and genus levels. The increase in Gammaproteobacteria was higher in mice that were pretreated with antibiotics prior to infection and was dose dependent for both the streptomycin- and vancomycin-pretreated groups. The treatment of mice with the intermediate and high doses of streptomycin and the high dose of vancomycin resulted in a statistically significant increase in the proportion of Gammaproteobacteria, compared to that in the untreated infected group (P < 0.05). An examination of infection-induced changes in the microbiota at the genus level demonstrated that the numbers of Enterobacteriaceae and culturable aerobic bacteria were increased in all of the examined antibiotic-pretreated infected groups and that the numbers of enterococci/group D streptococci were increased in the vancomycin-pretreated groups, compared to those in the untreated infected mice (Fig. 2B and C).

These data clearly indicate that the preinfection intestinal microbiota composition has an impact on the ability of the infectious agent to further interact with and modify the microbiota. More-significant perturbations in the microbiota of uninfected mice resulted in more-significant shifts when Salmonella serovar Typhimurium was introduced.
Antibiotic treatment does not significantly change the total numbers of intestinal microbiota but promotes infection-induced reduction in the total numbers of microbiota, concurrent with an increase in pathogen colonization. To investigate the reasons for alterations in postinfection microbiota composition observed by FISH and differential plating evaluation, we examined the total numbers of intestinal bacteria and Salmonella serovar Typhimurium colonization.

Infection with Salmonella serovar Typhimurium caused a dramatic reduction in the total numbers of microbiota of antibiotic-pretreated mice, compared to those of untreated infected mice ($P < 0.05$), in both colons and ceca (Fig. 3B and C). The total numbers of microbes in antibiotic-treated uninfected mice 3 days after the withdrawal of antibiotics did not differ significantly from the levels in untreated uninfected mice, except for the colonic microbiota of mice pretreated with 300 mg/liter of streptomycin and 100 mg/liter of vancomycin (Fig. 3B and C). The magnitude of the reduction in total numbers of microbiota as a result of infection (Fig. 3B and C) was associated with the extent of preinfection perturbation in microbiota composition (Fig. 1A). Moreover, greater Salmonella serovar Typhimurium intestinal burdens (Fig. 4) were associated with a greater infection-induced reduction in total numbers of microbiota (Fig. 3B and C). Therefore, the increased proportion of the Gammaproteobacteria in the microbiota of antibiotic-treated infected mice (Fig. 1B and C) was due to a combination of a decrease in total numbers of intestinal microbes in these mice (Fig. 3B and C) and increased colonization by Salmonella serovar Typhimurium (Fig. 4), which also belongs to Gammaproteobacteria. Consequently, the magnitude of the preinfection perturbation of the microbiota steady state was directly linked to the increased susceptibility of the host to Salmonella serovar Typhimurium colonization and to Salmonella serovar Typhimurium-induced perturbations of both the composition and total numbers of the microbiota.

The degree of Salmonella serovar Typhimurium-induced intestinal pathology correlates with the extent of preinfection microbiota perturbation. To examine how increased Salmonella serovar Typhimurium burdens and greater microbiota perturbations correlate with Salmonella serovar Typhimurium-induced intestinal disease, H&E-stained cecal necropsy specimens were examined. Ceca were chosen for histopathological evaluation, as they are the focal point of Salmonella serovar Typhimurium-induced intestinal pathology in mice. Tissues from uninfected mice, both untreated and antibiotic treated, showed no signs of intestinal disease, indicating that treatment with antibiotics alone did not induce a pathological response in the mouse intestinal tract (Fig. 5A). There were no signs of edema, no presence of inflammatory infiltrate, and no alterations in the epithelial architecture. When infected tissues were examined, signs of intestinal disease were observed in all but the untreated infected group (Fig. 5A). Mice pretreated with increasingly higher antibiotic doses prior to infection exhibited increasingly advanced pathology, starting with mild edema and minimal alterations to the epithelial layer and progressing to copious inflammatory infiltrate, severe edema, and complete loss of epithelial architecture. Although all mice infected with Salmonella serovar Typhimurium appeared sick (ruffled fur and sluggish movements) at the time of sacrifice due to Salmonella serovar Typhimurium systemic infection (Salmonella serovar Typhimurium was present in the spleens...
of all infected mouse groups [data not shown]), mice with more extensive intestinal pathology were more moribund (seemingly more lethargic) than those in other infected groups.

Pathological scoring (9) demonstrated that while tissues from uninfected animals were healthy (minimal pathology scores), *Salmonella* serovar Typhimurium-infected animals showed overt signs of intestinal pathology that correlated with the extent of preinfection microbiota perturbation (Fig. 5B).

Pathological indices were increased in all of the tissue sections of antibiotic-pretreated infected mice, becoming significantly greater than those of the untreated infected group (*P* < 0.05).

**The severity of typhlitis in antibiotic-treated mice correlates with the levels of inflammatory cytokines in the diseased ceca.**

To evaluate the infiltrating inflammatory cells in cecal inflammation (typhlitis), a panel of four inflammatory cytokines and chemokines was examined: TNF-α, MCP-1, keratinocyte chemotactant, and interleukin-6. Together, these inflammatory mediators function to activate and/or attract a wide array of inflammatory cells to the site of inflammation, leading to the characteristic histopathological changes observed in typhlitis.

**FIG. 5.** *Salmonella* serovar Typhimurium-induced intestinal pathology. Mice were treated with the specified antibiotics in drinking water for 2 days. Doses are in mg/liter. Following antibiotic withdrawal, mice were infected with $2.7 \times 10^8$ CFU of *Salmonella* serovar Typhimurium for 3 days. Experiments were repeated three times with six mice per group. The results of a representative experiment are shown. *P* values were calculated using Kruskal-Wallis with Dunn’s posttest, with a 95% confidence interval. C, control; Sm, streptomycin; Vanc, vancomycin. (A) Tissues were harvested, fixed in formalin, and stained with H&E. Antibiotic-treated *Salmonella* serovar Typhimurium-infected sections show escalating pathology, indicated by rising levels of inflammatory infiltrate starting from the submucosa and spreading to the lumen, as well as increasing epithelial disorganization, indicated by mucinous plugs in crypts, mounting epithelial regenerative changes, desquamation, and the presence of dead epithelial cells in the lumen. Arrowheads indicate the lumen. (B) Quantification of indicators of pathology. Groups marked with an asterisk are significantly different from the respective C group (*P* < 0.05).
inflammatory cells to the site of injury or infection, such as macrophages, neutrophils, and T and B cells (1, 19, 20), and their presence can therefore be used as an indication of the inflammatory infiltrate.

None of the uninfected ceca had elevated levels of any of the examined inflammatory mediators present (Fig. 6). Thus, antibiotics alone did not induce an inflammatory milieu in the intestinal tract of the treated mice. Similarly, untreated infected ceca had negligible levels of all of the examined inflammatory mediators, consistent with the fact that regular mouse microbiota provides its host with protection from *Salmonella* serovar Typhimurium-induced intestinal disease. Conversely, ceca of infected mice that have been pretreated with streptomycin or vancomycin had high levels of all the examined inflammatory mediators, indicating that more-severe typhlitis observed in cecal necropsy specimens (Fig. 5) was due to increased inflammatory infiltrate in the infected tissues. The increase in inflammatory mediators was dependent on the dose of antibiotics that mice received prior to infection and consequently on the extent of preinfection microbiota perturbation.

**DISCUSSION**

Intestinal microbiota is the collection of microorganisms inhabiting the intestinal tract of the host. Numerous functions of the microbiota and its contributions to the physiology and anatomy of the intestinal tract make these bacteria an invaluable partner to their eukaryotic host. However, despite our appreciation of the microbiota’s importance, the specifics of its contributions to various disease states are not well understood. Moreover, the effect of microbiota perturbations on predisposition to and progression of various diseases is not well known.

While examining the effects of antibiotics on the intestinal microbiota, we were surprised to see that although vancomycin’s spectrum of activity is exclusively gram-positive bacteria (33), gram-negative bacteria (CFB phylum) appeared to be adversely affected by the treatment. Perhaps certain gram-positive bacteria that were resistant to the effects of vancomycin were able to dominate the niches of their more sensitive relatives, as a result making the overall colonic environment more hostile to the members of the CFB phylum. However, vancomycin treatment promoted the proliferation of gram-negative bacteria of the *Gammaproteobacteria* phylum, specifically the *Enterobacteriaceae*. The main effect of streptomycin on microbiota was in promoting the growth of CFB bacteria at the expense of other phyla. As this effect was dose dependent, it was likely due to a selective inhibition of a microbial population whose vacated niche CFB organisms were then able to colonize.

After infection with *Salmonella* serovar Typhimurium, it was observed that the pretreatment of mice with increasing doses of vancomycin resulted in an increase in the proportion of...
Gammaproteobacteria postinfection. A higher variability in response to Salmonella serovar Typhimurium was observed in infected mice treated with the low vancomycin dose than in those treated with the higher vancomycin dose. Streptomycin-pretreated mice exhibited a more uniform response to Salmonella serovar Typhimurium infection than vancomycin-pretreated mice. This could be due to the fact that streptomycin, unlike vancomycin, did not encourage the growth of commensal Enterobacteriaceae, thus preventing the potential competition between them and Salmonella serovar Typhimurium. Bacteria of the CFB phylum were more adversely affected during infection with Salmonella serovar Typhimurium than bacteria in Firmicutes and other phyla (Fig. 1B and C), a result that is similar to that noted by Stecher et al. during Salmonella serovar Typhimurium infection in a high-dose streptomycin pretreatment model (40). The higher sensitivity of bacteria belonging to the CFB phylum could be due to the inflammatory changes observed during Salmonella serovar Typhimurium infection. The presence of reactive oxygen species during inflammation could be detrimental to the strictly anaerobic CFB bacteria.

The increased susceptibility of mice to Salmonella serovar Typhimurium infection as a result of treatment with selected antibiotics could be due to a number of factors, such as alterations in the host immune response due to a disturbance in the microbiota (as it is known that the microbiota contributes to the establishment of intestinal immunity) (29), a selective removal of a group of bacteria that usually provide a barrier to Salmonella serovar Typhimurium colonization and/or persistence, or a combination of these two options. At least two groups of bacteria, lactobacilli and enterococci/group D streptococci, were selectively inhibited by both antibiotics used in the study. Both of these bacteria belong to the gram-positive Firmicutes phylum, and it has previously been shown in vitro that gram-positive fecal isolates are better able than gram-negative ones to inhibit the growth of Salmonella serovar Typhimurium (15). Further studies focusing on detailing the effects of antibiotics on different species making up the microbial community could shed more light on which members are specifically needed for protection against various invading pathogens. To date, most studies on the contribution of particular members of the bacterial population to the inhibition of enteric pathogens focused on probiotic bacteria in epithelial cell models (8, 10, 15). Our studies highlight the importance of the microbiota in host response to infection in an animal model and identify potentially important groups of microbiota that could become the focus of future studies.

Greater Salmonella serovar Typhimurium burdens in mice treated with increasing doses of antibiotics were also associated with more-profound intestinal inflammation and pathology, as well as with greater postinfection alterations in microbiota, as evidenced by both a reduction in total numbers of bacteria and a larger proportion of Gammaproteobacteria making up the intestinal microbiota. The enhanced intestinal inflammation and pathology were likely due to a greater activation of the host immune system by higher numbers of Salmonella serovar Typhimurium. The cytokine profile was indicative of infiltration by neutrophils and macrophages, which is consistent with the current knowledge of the innate immune response to Salmonella serovar Typhimurium infection (44). Additionally, the cytokine profile was reminiscent of that observed for inflammatory bowel diseases (increases in TNF-α and MCP-1) (3, 38), which are also characterized by disturbances in intestinal microbiota (13), particularly an increase in colonization by Gammaproteobacteria. We have previously shown that a strong inflammatory response acts to reduce the total numbers of intestinal microbiota (27), which is likely the reason for a reduction in total numbers of bacteria observed postinfection. As well, a strong inflammatory response was shown to benefit the growth of enteropathogens (27, 40) and nonpathogenic aerobic bacteria (27). Salmonella serovar Typhimurium-induced inflammation was previously shown to adversely affect the members of murine cecal microbiota, promoting the overgrowth of the pathogen (40), which is also confirmed by our results.

Most previous studies looking at murine Salmonella serovar Typhimurium-induced colitis utilize extremely high doses of antibiotics (20 mg streptomycin/mouse) (5, 7), eliminating 90 to 98% of the intestinal microbiota prior to infection (40). Thus, it was ambiguous which component of the antibiotic-induced perturbation of the microbiota was responsible for the disruption of resistance to Salmonella serovar Typhimurium colitis: a change in the composition of the normal flora or the reduction in total numbers of bacteria. We have shown that a perturbation in the composition alone is sufficient to increase the susceptibility of the murine host to Salmonella serovar Typhimurium colitis. A recently published study (23) has also found that even when bacterial numbers returned to normal levels after antibiotic treatment, mice still remained more susceptible to Salmonella serovar Typhimurium infection. This study, however, did not examine the shifts in microbiota composition either pre- or postinfection and did not conclusively demonstrate the correlation between the perturbations in microbiota composition and increased susceptibility to Salmonella serovar Typhimurium.

Our results demonstrate profound perturbations in the composition of the intestinal microbiota as a result of antibiotic treatment. Furthermore, they show that microbiota imbalance predisposes the host to more severe enteropathogenic infection. These observations could be part of the explanation for the high rates of nosocomial infections, where antibiotics are abundantly used. In fact, nosocomial Salmonella enterica infections, particularly with multidrug-resistant strains, are a concern in developing countries (22, 42) and occasionally even happen in developed countries (18, 33). Although our findings cannot be indiscriminately extrapolated to all diseases where microbiota imbalance has been implicated in the etiology, they show that, at least in infectious colitis, microbial imbalance precedes the onset of pathology rather than being the result of it. Consequently, the initiation of pathology could be averted or corrected by maintaining a balanced microbial community.

Antibiotic usage is extensive in our society, both in hospitals and in the community. However, the effect of antibiotics on the intestinal microbiota has not been extensively scrutinized other than in studies on the induction of antibiotic resistance in commensal bacteria following treatment. The demonstrated perturbations in the microbial community as a result of antibiotic treatment warrant further detailed investigation of the effects of the most frequently used antibiotic regimens on the
composition of probiotic supplements and attempts to find probiotic or prebiotic supplements that would offset these perturbations.

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