Indigenous microbes inside the host intestine maintain a complex self-regulating community. The mechanisms by which gut microbes interact with intestinal pathogens remain largely unknown. Here we identify a commensal *Escherichia coli* strain whose expansion predisposes mice to infection by *Vibrio cholerae*, a human pathogen. We refer to this strain as ‘atypical’ *E. coli* (atEc) because of its inability to ferment lactose. The atEc strain is resistant to reactive oxygen species (ROS) and proliferates extensively in antibiotic-treated adult mice. *V. cholerae* infection is more severe in neonatal mice transplanted with atEc compared with those transplanted with a typical *E. coli* strain. Intestinal ROS levels are decreased in atEc-transplanted mice, favouring proliferation of ROS-sensitive *V. cholerae*. An atEc mutant defective in ROS degradation fails to facilitate *V. cholerae* infection when transplanted, suggesting that host infection susceptibility can be regulated by a single gene product of one particular commensal species.
Commensal microbes, collectively termed gut microbiota, are considered to exist as a symbiotic community in the mucus layer lining the intestinal epithelium. Due to the lack of means to isolate and preserve intestinal tissues, how these microbes maintain a dynamic and permanent co-evolutionary relationship with the host is not clearly understood. In some imaging studies, however, biofilm-like structures were successfully observed in animal intestines and surgically removed human appendices. This suggests that (i) the resident microbes can act as a barrier against invading pathogens and (ii) enteric infections are the outcomes of multifaceted interactions between commensals, pathogens, and the host intestinal tissue.

Infectivity of pathogenic *E. coli* strains is controlled by the composition of commensal *E. coli* strains that can metabolize specific carbohydrates, thereby reducing their availability for consumption by pathogenic strains. Anaerobic growth of both *E. coli* and *Salmonella enterica* serovar typhimurium can be supported by respiration using nitrate or tetrathionate, which are byproducts of the host inflammatory response. Certain bacterial pathogens can increase their survival fitness inside the host intestine by catabolizing host-derived carbohydrates, the production of which is mediated by *Bacteroides thetaiotaomicron*, a distinct member of the gut microbiota. The alteration of gut microbiota composition by antibiotic treatment has been shown to increase host susceptibility to intestinal infections by *S. enterica* and *Clostridium difficile*. Furthermore, individuals for whom faecal transplantation resulted in restored microbiota have been shown to exhibit improved resistance to recurrent *C. difficile* infection. However, due to the high degree of diversity of commensal microbes and the difficulty in culturing these strains, it has been difficult to correlate host susceptibility to infection with changes in the relative abundance of a defined subgroup of commensal microbes.

In this study, we seek to identify a commensal species and its genetic factor(s) that specifically influence host resistance to enteric infection. An *E. coli* strain with unusual features was significantly propagated during antibiotic treatment in mice and found to be responsible for modulating host susceptibility to infection by *V. cholerae* (*Vc*). We sequenced the whole genome of the *E. coli* strain and identified a novel catalase gene, disruption of which abrogated the infection-facilitating effects. This report provides novel insights into the role of gut microbiota in regulating the extent of intestinal infections.

### Results

**Antibiotic-treated mice became sensitive to *Vc* colonization.** Commensal microbes play protective roles in host immunity against enteropathogenic infections. To examine whether host susceptibility to intestinal infection is influenced by altered gut microbiota composition, we treated adult mice with low concentrations of streptomycin (SM, 1 mg per day) or vancomycin (VAN, 250 μg per day) daily for 7 days (Fig. 1a). Fluorescence staining of microbial cells recovered from mouse faeces revealed varying numbers of bacterial cells with differential shapes, with slightly more bacterial cells found in faeces obtained from SM-treated mice (Fig. 1b). Aerobic cultivation of mouse intestinal homogenates in plate count agar medium revealed that more bacterial colonies grew from faeces obtained from SM-treated and VAN-treated mice compared with nontreated mice (Fig. 1c). These results suggest that our experimental conditions induced a change in the gut microbiota composition, rather than eliminating considerable portions of commensal microbes.

The adult mouse is not a natural host for *Vc*, the causative agent of the pandemic human disease cholera. When control mice were infected with *Vc* via oral gavage, no significant *Vc* colonization was observed (Fig. 1d). However, a marked increase in *Vc* colonization was observed in antibiotic-treated mice; bacterial colonization was increased ~100-fold and ~10,000-fold in SM-treated and VAN-treated mice, respectively (Fig. 1d). We then measured the relative amount of the *Vc* 16S ribosomal RNA (rRNA) gene in the small intestine of each group. Consistent with the increased *Vc* colonization, a higher level of the *Vc* gene was detected in antibiotic-treated mice and this increase was most significant in VAN-treated mice (Fig. 1e). Altogether, these results demonstrate that administration of low concentrations of antibiotics alters the mouse gut microbiota composition, which subsequently results in increased host vulnerability to *Vc* colonization.

**An atEc strain proliferated on antibiotic treatment.** We then quantified the relative abundance of different commensal microbial phylogenetic groups in each treatment group by real-time PCR using group-specific primer sets (Supplementary Table 1). Genomic DNA extracted from small intestine tissue lysates was subjected to amplification and normalized to the level of the host *gapdh* gene. The relative quantity of the γ-proteobacteria 16S rRNA genes was markedly increased in VAN-treated mice (Supplementary Fig. 1a). Subsequent family-level and species-level examinations indicated that the levels of the Enterobacteriaceae and *E. coli* 16S rRNA genes were remarkably increased in VAN-treated mice and were also increased, albeit to a lesser extent, in the SM-treated group (Supplementary Fig. 1b,c). In contrast, the relative quantities of the marker genes for selective detection of *Bifidobacterium*, *Lactobacillus* and *Bacteroides* were similar between all groups (Supplementary Fig. 1d–f). These results demonstrate that (i) the abundance of *E. coli* was specifically increased during treatment with SM or VAN, and (ii) this increase likely accounted for the elevated levels of Enterobacteriaceae and γ-proteobacteria class bacteria in the antibiotic-treated groups.

Next, suspensions of mouse faeces were cultivated on eosin methylene blue (EMB) agar, a selective medium for Gram-negative bacteria, especially those belonging to the Enterobacteriaceae family. Consistent with our DNA-based assay results, more colonies grew from faeces obtained from SM-treated and VAN-treated mice (Fig. 2a). Species identification was performed on several colonies and revealed that all were *E. coli*. This finding further supports the results shown in Supplementary Fig. 1. Intriguingly, however, a majority of the bacterial cells grew as colourless colonies on the EMB plates (Fig. 2a). Typical *E. coli* strains can ferment lactose, thereby yielding colonies with a distinctive metallic green sheen when grown on EMB plates. As shown in Fig. 2b, the numbers of atypical (that is, colourless) *E. coli* (termed atEc) colonies increased to ~10³ and ~2.5 × 10⁴ per g of faeces in SM-treated and VAN-treated mice, respectively. Notably, typical green colonies (termed tEc) were also recovered in the VAN-treated group (Fig. 2b). We then performed an RAPD assay to examine the extent of genomic diversity among these *E. coli* isolates. Sixteen strains (14 colourless, 1 green and 1 intermediate green) were included in this analysis. Although the amplification products were almost identical among the atEc strains, these products were distinct from those obtained from the typical green and intermediate green strains (Supplementary Fig. 2). This result suggests that (i) atEc strains possess a distinct genome, and (ii) the antibiotic-induced propagation of atEc strains was likely mediated by clonal expansion. The numbers of atEc strains were also significantly increased in the small intestine and the colon of SM-treated and VAN-treated mice (Fig. 2c,d). In each group, atEc strains were more prevalent in the colon than in
the small intestine, indicating that the colon is a more suitable habitat for this strain.

We next asked whether propagation of the atEc strain was invariably observed in mice that received a different antibiotic regimen (Supplementary Fig. 3a). Treatment of adult mice for 4 weeks with a mixture of four different antibiotics in the drinking water revealed a similar increase in the number of colourless colonies, which were later identified as *E. coli*, on EMB plates (Supplementary Fig. 3b). Of particular note, the mean mouse colon weight was increased in the antibiotic-treated group (0.2 g) versus the control group (0.16 g), an apparent host phenotypic change in response to antibiotic treatment (Supplementary Fig. 3c). On subsequent *Vc* infection, antibiotic-treated mice exhibited significantly increased fluid accumulation (FA) in their small intestines (Supplementary Fig. 3d). The extent of FA has been shown to depend on the degree of *Vc* infectivity of the host. Most importantly, ~50-fold more *Vc* cells were recovered from the intestines of antibiotic-treated mice (Supplementary Fig. 3e). Altogether, these findings further suggest that the atEc strain is highly proliferative under antibiotic stress and that *Vc* infection is facilitated by the increased abundance of the atEc strain.

**The atEc strain possesses an extra catalase gene.** We next sought to understand the molecular basis underlying the positive effect of the atEc strain on *Vc* colonization. To this end, we sequenced the genomes of the atEc and tEc strains and compared them with the *E. coli* K12 genome. The atEc strain was found to have the largest genome (5.24 Mbp), whereas the tEc and K12 genomes were 4.72 Mbp and 4.64 Mbp, respectively (Supplementary Fig. 4a). Alignment of the atEc and tEc genomes using the Maximal Unique Matcher algorithm revealed that most of the regions overlapped with each other (Supplementary Fig. 4b, red diagonal line). However, many similar genetic elements were found to be highly scattered over the entire genomes, revealing a high degree of dissimilarity between the two genomes (Supplementary Fig. 4b). Of particular note, the 3′ region of the lacY gene (which encodes lactose permease) was deleted in the atEc strain, whereas the full-length lacY gene was detected in the tEc and K12 strains (Supplementary Fig. 4c). The presence of a defective lacY gene explains why the atEc strain formed colourless colonies on the lactose-containing EMB plates.

We then carried out genome-based clustering analysis using two isolates and representative *E. coli/Shigella* strains. In a dendrogram constructed based on the average nucleotide identity (ANI), the tEc and atEc strains were quite distant from *E. coli* K12 (Supplementary Fig. 5). Furthermore, tEc and atEc were relatively distant from each other, with ANI of 99.1%, indicating that the two strains belong to different phylogenetic lineages within the *E. coli* group (Supplementary Fig. 5).

Importantly, the comparison with the tEc genome showed that the atEc genome contains an extra gene encoding a catalase, a critical enzyme that protects cells from ROS-mediated oxidative...
Serratia proteins from unrelated species (for example, of the genera eKatE. In contrast, proteins from both strains (Supplementary Fig. 7b, black arrows). Likewise, evolutionary distance was not detected between KatE (Supplementary Fig. 6d). This information suggests that the eKatE gene might have been horizontally transferred from another species with a different degree of relatedness.

To provide supporting evidence for horizontal transfer of the eKatE gene, we performed several bioinformatics analyses. First, we constructed two different phylogenetic trees based on amino-acid sequences of KatG and KatE proteins, respectively (Supplementary Fig. 7). In the KatG-based tree, KatG proteins from tEc and atEc strains were clustered together with those of other E. coli strains (Supplementary Fig. 7a, black arrows). Likewise, evolutionary distance was not detected between KatE proteins from both strains (Supplementary Fig. 7b, black arrows). In contrast, eKatE-encoded catalase was distinctly clustered with proteins from unrelated species (for example, of the genera Serratia, Frankia, Lonsdalea and Rouxiella; Supplementary Fig. 7b, red arrow). Second, tetranucleotide frequency was analysed for the katE genes found in 33 representative E. coli strains. The tetranucleotide frequency of the eKatE gene exhibited the lowest correlation coefficient when compared with other values in the matrix table (Supplementary Table 2). In contrast, katE genes of the atEc or tEc strain were similar to other katE genes of E. coli origin in terms of the tetranucleotide frequency.

Third, we also measured codon adaptation index (CAI) of three catalase genes (katG, katE and eKatE) of the atEc strain. The CAI of the eKatE gene clearly deviated from that of the other two genes (Supplementary Table 3). Moreover, GC content of the eKatE gene was significantly lower than that of katG and katE genes. Fourth, shared synteny was observed for genes encoding transposase in tEc and E. coli K02 strains (Supplementary Fig. 8). Genes encoding transposases are also detected in Serratia and Frankia species (Supplementary Fig. 8). More importantly, ccdBA genes are present near the eKatE gene in the atEc chromosome (Supplementary Fig. 9). These two genes are known to be plasmid-borne and produce the CcdA/CcdB toxin-antitoxin module that is involved in plasmid maintenance in E. coli. Altogether, these analyses strongly suggest that the atEc strain acquired the eKatE gene by horizontal gene transfer from an outside mobile genetic source.

The atEc strain is highly resistant to H2O2. On the basis of our native gel-based activity assay (Supplementary Fig. 6b), eKatE-encoded catalase appeared to have a stronger enzymatic activity than KatG or KatE, suggesting that the atEc strain might be more resistant to H2O2 than the tEc strain. In LB supplemented with 2 mM H2O2, atEc cells grew completely normally, as they did in plain LB (Fig. 3a). In contrast, growth of the tEc strain was significantly inhibited in the presence of H2O2 (Fig. 3a).
Bacterial cultures were spot-inoculated onto LB plates. (a) Overnight cultures of N16961 cells were diluted 100-fold in LB + 2 mM H2O2 that had been preincubated with atEc for 2 h, whereas no growth was observed in the same medium that had been pre-treated with tEc for the same period of time (Fig. 3b). These results suggest that atEc cells can detoxify H2O2, thereby helping Vc cells proliferate in the presence of ROS stress. An additional 18 strains that formed colourless colonies on the EMB plates also produced significant levels of eKatE, further supporting the idea that the atEc strain had clonally expanded during antibiotic treatment (Supplementary Fig. 10). The relative quantity of the eKatE gene in small intestine tissue lysates was increased in the SM-treated group and to an even greater extent in the VAN-treated group (Supplementary Fig. 11). The increase in the level of the eKatE gene was proportional to that of the E. coli 16S rRNA gene (Supplementary Fig. 1c). This result further indicates that the increased population of E. coli in antibiotic-treated mice can be attributed to the proliferation of atEc strains.

The tEc strain also produces KatG and KatE, two distinct catalases (Supplementary Fig. 6b). To further verify the role of eKatE in H2O2 resistance, we constructed recombinant tEc and Vc strains that express a plasmid-borne or a chromosomally inserted eKatE gene, respectively. A DNA element encompassing both the eKatE gene open reading frame and its endogenous promoter was used for cloning. The recombinant tEc strain harbouring the pBAD24:eKatE plasmid was found to be resistant to 2 mM H2O2 and grew completely normally in the presence of 2 mM H2O2, as in plain LB media (Fig. 3c). Moreover, a Vc N16961 strain expressing the eKatE gene was equally resistant to 2 mM H2O2 (Fig. 3d). These results demonstrate that the eKatE-encoded catalase is responsible for the ROS resistance detected in atEc cells. Besides the unique presence of eKatE gene in the atEc strain, genetic repertoires for oxidative stress responses are almost identical between the two strains. In each strain, 57 genes were identified that are known or presumed to be involved in oxidative stress response. Among these, 56 genes are found in both genomes, except for atEc_eKatE and tEc_eKatE genes (Supplementary Table 4).

**Vc infection is severe in the atEc-transplanted infant mice.** Infant mice have been widely used as a surrogate host to study Vc infection in vivo. We found that antibiotic-treated mice exhibited a substantially altered gut microbiota composition and increased susceptibility to Vc colonization. To define causality in the relationship between the increased intestinal population of atEc and increased host sensitivity to Vc infection, infant mice were challenged with Vc following daily transplantation with 10⁷ atEc or tEc cells for 3 days (Fig. 4a). Transplantation of tEc or atEc was efficiently achieved, as demonstrated by the recovery of ~4 × 10⁷ tEc and ~9 × 10⁷ atEc cells from mouse intestines at 3 days post transplantation (Fig. 4b). Significantly lower bacterial
are expressed as means ± s.e.m. and are displayed on a log scale. ***P<0.001 versus the number of bacterial colonies from the control group. (c) Mouse intestinal tissue lysates obtained from each group (n = 4) were stained with 100 μM PO1 for 30 min to detect ROS. Values are expressed as means ± s.e.m. and are displayed in each bar. *P<0.05 versus PO1 signals from the ‘before Vc infection’ groups. **P<0.01 versus the signals of all the other groups. (d) Each group of mice was challenged with Vc (n = 7, left panel) or left uninfected (n = 7, right panel). The fluid accumulation (FA) ratio of each group was measured and plotted on a linear scale. *P<0.05 versus PO1 signals from the ‘before Vc infection’ groups. **P<0.01 versus the FA ratio of the Vc-infected control group. ***P<0.01 versus the FA ratio of the Vc-infected tEc-transplanted mice. *** indicates the FA ratios of the noninfected groups were significantly different from those of all Vc-infected groups (P<0.005). (e) The number of Vc cells that colonized the mouse intestine (n = 7 in each group) was determined by CFU counting. Values are expressed as means ± s.e.m. and are displayed on a log scale. *P<0.05 versus Vc CFUs of the control or tEc-transplanted group. (f) Infant mice (n = 7 per group) were infected with a Vc ctxAB promoter fusion reporter strain. The level of bioluminescence was measured in each mouse intestinal tissue homogenate. Values are expressed as means ± s.e.m. and are displayed on a linear scale. *P<0.05 versus the bioluminescence level in control mice.

Increased Vc infection is due to eKatE-encoded catalase. We then examined whether the catalase activity of the eKatE gene product plays a role in atEc-mediated enhancement of Vc infection. To address this, we constructed an eKatE in-frame deletion mutant of atEc strain. The data shown in Fig. 5a clearly demonstrate the lack of eKatE-encoded catalase activity of the mutant. Disruption of the eKatE gene abrogated the H2O2 resistance of the atEc strain (Fig. 5b). The atEc ΔeKatE mutant failed to protect Vc cells against H2O2 stress in our in vitro co-culture system (Fig. 5c). Importantly, the extent of Vc infection-mediated FA induction was significantly reduced in ΔeKatE-transplanted infant mice compared with atEc-transplanted infant mice (Fig. 5d). Moreover, the facilitatory effect of atEc transplantation on Vc colonization disappeared when the eKatE gene was deleted. As shown in Fig. 5e, 10-fold higher numbers of Vc cells were recovered after 24-h infection in atEc-transplanted infant mice, compared with tEc- or ΔeKatE-transplanted groups. It is of particular interest that atEc remained colonized during the 24-h Vc infection period, whereas the abundance of tEc and the ΔeKatE mutant substantially decreased in response to Vc infection (Fig. 5f). These findings suggest that (i) the atEc strain possesses better ‘colonization fitness’ under Vc infection-induced host stress conditions and (ii) Vc in vivo colonization likely occurs as a consequence of co-operative interaction with atEc cells in a catalase-dependent manner.
Finally, we examined whether the H₂O₂-resistant Vc strain shown in Fig. 3d exhibited a superior colonization capability in conventional adult mice. The N16961::pVIK112+ eKatE strain colonized significantly better than the control strain; at 8 h post-infection, ~10,000-fold more bacterial cells remained colonized in the mouse small intestine (Fig. 5g). Although lower numbers of bacterial cells were recovered after 16 h of infection, the eKatE-expressing cells still colonized ~13.8-fold better than the control cells (Fig. 5g). This result demonstrates that Vc colonization also occurs more readily when Vc alone can handle ROS stress.

Discussion
The gut microbiota and products encoded by its genome (that is, the gut microbiome) play critical roles in human health. Commensal bacteria that reside in the host intestine contribute to the development of a functional intestinal immune system. The gut microbiome, which is considered to supplement the human genome with >100-fold more genes, contains genes that produce digestive enzymes lacking in humans. Furthermore, phylum-level changes in their composition account for the differential propensity of individuals to develop obesity. Along with these important functions, commensal microbes also participate in regulating host defenses against the invasion of pathogenic bacteria. In this study, we isolated a commensal E. coli strain that exhibits exceptional resistance to ROS and demonstrated that such an atypical E. coli strain, when abundantly present in the host intestine, can enhance host susceptibility to enteric infection.

Our results suggested that the atEc strain was slightly more resistant to SM than the tEc strain; in the presence of SM (32 μg ml⁻¹), atEc cells grew slightly better than tEc cells (red arrow, Supplementary Fig. 12). However, both atEc and tEc strains were equally sensitive to ampicillin and tetracycline (data not shown). In addition, both strains showed no sensitivity to VAN, an effective antibiotic against Gram-positive organisms (data not shown). On the basis of these results, it appears that the atEc strain is not more resistant to antibiotics and therefore the abundant population of atEc in antibiotic-treated mice is not caused by its superior capability to survive antibiotic treatment.

On the other hand, host-mediated changes may play a critical role in creating an environment that stimulates the propagation of atEc cells in antibiotic-treated mice. Accumulating evidence suggests that an increase in LPS inside the host intestine stimulates ROS production. During antibiotic treatment, the leve of Enterobacteriaceae, a large family of Gram-negative bacteria, was greatly increased. Therefore, it is likely that the amount of LPS released from those Gram-negative species may...
also be significantly increased. Consistent with this notion, the data in Fig. 4c show that the PO1 signal was noticeably increased in E. coli-transplanted mice, indicating that the increased population of E. coli cells inside the mouse intestine resulted in elevated ROS levels. Stable transplantation of the atEc strain, however, resulted in a significant decrease in the level of ROS inside the host intestine (Fig. 4c). This change in the intestinal environment presumably helps Vc, which is known to be ROS-sensitive, colonize and exert pathogenic effects. Recently, Lupp et al. reported that the relative abundance of nonpathogenic E. coli was increased during chemically induced intestinal inflammation, a process that generates excessive ROS. However, no basis was provided for such a population change. It will be very interesting to see whether the E. coli strain described in their study shares common phenotypes with the atEc strain that we identified in the current study. The robust activity of the eKatE-encoded catalase helped the atEc strain overcome surrounding environmental changes, which likely involved the accumulation of high levels of ROS. Although it remains unclear how much ROS was actually produced during antibiotic treatment in our model system, the atEc strain must have competitive survival fitness under conditions of host-mediated ROS production.

Vc colonization and cholera toxin-induced intestinal fluid accumulation were increased in atEc-transplanted infant mice, but not in mice transplanted with atEc cells lacking the eKatE gene. These results indicated that the pathogenesis of Vc infection was critically influenced by gut microbiota composition and a microbiome gene product that regulates intestinal ROS level in vivo. Diverse virulence determinants have been reported to play distinct roles in V. cholerae pathogenesis in different animal models. For example, persistent colonization of Vc in adult mice depends on accessory toxins (that is, hemolysin and RTX toxin), but not on toxin co-regulated pilus. In addition, cholera toxin promotes bacterial colonization in infant mice and infant rabbits. In adult mice, Vc colonization and cholera toxin-induced intestinal fluid accumulation were increased in atEc-transplanted infant mice, but not in mice transplanted with atEc cells lacking the eKatE gene. These results indicated that the pathogenesis of Vc infection was critically influenced by gut microbiota composition and a microbiome gene product that regulates intestinal ROS level in vivo. Diverse virulence determinants have been reported to play distinct roles in V. cholerae pathogenesis in different animal models. For example, persistent colonization of Vc in adult mice depends on accessory toxins (that is, hemolysin and RTX toxin), but not on toxin co-regulated pilus. In addition, cholera toxin promotes bacterial colonization in infant mice and infant rabbits. These previous results strongly suggest that host age is an important factor that affects susceptibility to Vc infection. Since changes in microbiota composition are clearly observed with aging, how Vc infectivity is modulated by age-dependent changes in microbiota composition must be further addressed.

The development of cholera vaccines has been hampered, in part, by the difficulty in assessing the efficacy of a candidate vaccine economically using adult mice. Our results suggest that adult mice with an increased population of atEc cells can develop cholera-like symptoms. The activity of catalase encoded by the eKatE gene appears to be significantly higher than that of other well-characterized catalases. We showed that a recombinant Vc strain that produces eKatE-encoded catalase was fully resistant to 2 mM H2O2 and this ROS-resistant Vc strain exhibited enhanced colonization capability in a normal adult mouse. Provided that virulence is unaffected by eKatE gene expression, this recombinant Vc strain may have potential application as a challenge strain in future vaccine development.

As shown in Supplementary Data 1, 269 different katE-encoded catalases were identified in the human gut microbiome catalogue. This indicates that at least 269 bacterial species can produce proteins identical or similar to the KatE catalase in human intestine. Of note, KatE proteins produced by the species belonging to the Proteobacteria phylum exhibit the highest sequence similarities with that of the atEc strain. The genomes of 50 bacterial species, mostly in the same phylum, encode KatG catalases. Meanwhile, in the mouse gut microbiome catalogue, nine and seven katE- and katG-encoded catalases were identified, respectively. Interestingly, Proteobacteria is not a major group that produces KatE catalase in mouse intestine. Altogether, these results suggest that KatE might be produced in larger quantities than KatG in both human and mouse intestines, and KatE encoding catalase contributes to the production of KatE catalases in human versus mouse intestine. Currently, detailed information is lacking in regards to the relative abundances of individual catalase producers in the gut microbiota, and more information is needed to more definitively compare overall catalase activity in human versus mouse intestines. We expect that such information would prove useful in identifying the mechanisms of human-restricted tropism of bacterial infections, including that by V. cholerae.

The commensal species that modulate host susceptibility to disease are beginning to be defined. However, the underlying genetic determinants that modulate this susceptibility have not yet been identified. The ability of atEc to facilitate Vc infection was completely abrogated by the deletion of a single gene, eKatE. This finding demonstrates that the level of ROS inside the host intestine, which must be carefully regulated for protection against pathogenic invaders, can be controlled by a single-microbiome gene product. We anticipate that our results (summarized in Fig. 6) will stimulate the assembly of a database of microbiota-associated genes with defined functions, to better understand the roles such genes play in the complicated ecosystem of the host intestine.

**Methods**

**Bacterial strains.** The indigenous E. coli strains termed typical E. coli (tEc) and atypical E. coli (atEc) were isolated from CD-1 mouse intestines. V. cholerae (Vc) O1 serotype N16961 (ref. 44) was used as a model pathogen in all experiments. All strains were routinely grown aerobically in Luria-Bertani (LB) broth (10 g tryptone, 5 g yeast extract, and 10 g NaCl per l) or on LB agar plates (15 g l−1 agar) at 37 °C. Streptomycin (200 μg ml−1) was added to selectively cultivate N16961.

![Figure 6](image-url) **Figure 6** | **Summary of antibiotic-induced proliferation of atEc and its impact on host susceptibility to Vc infection.** Phylogenetically diverse bacterial cells that were originally present in the untreated host are depicted in different colours and shapes. Defective and enhanced colonization of Vc cells are denoted with dotted and solid lines (comma-shaped), respectively. The increased population density of atEc is indicated by the increased number of purple cells.
Construction of mutant and reporter strains. The atEc ΔekateE knockout mutant was created by allelic replacement as described previously. The 500-base pair flanking regions of both ends were amplified by PCR from each plasmid and ligated into the G-Spin genomic DNA. The constructed plasmid was then transformed into E. coli DH5α (KanR) by electroporation. Bacteria were grown on LB plates and the colonies were picked and cultured in LB for 18 h at 37°C. The genomic DNA from transformants was extracted and used to screen for the correct insertion and orientation by colony PCR. The correct colonies were then grown in LB medium at 37°C for 15–16 h with shaking under aerobic conditions. Ten microliters of genomic DNA was used as the template in the PCR reaction. The specific primers for the selection marker and the target fragment were used to screen the positive clones.

Mouse models and housing conditions. Experiments were performed with CD-1 and BALB/c mice purchased from Oriental Bio (Seoul, Korea). Pregnant BALB/c mice were purchased from Central Lab. Animal Inc. (Seoul, Korea) and raised for neonatal delivery. Mice were provided with irradiated food and sterile water. All animal studies were performed in compliance with the guidelines provided by the Department of Animal Resources of Yourense Biomedical Research Institute. The Committee on the Ethics of Animal Experiments at the Yonsei University College of Medicine approved this study (permit number 2011-0166). For antibiotic treatment, 5- to 6-week-old CD-1 female mice were orally treated with streptomycin (250 mg), or PBS as a control once a day for 7 days. Antibiotic dosages were chosen to induce alteration of the gut microbiota composition without eliminating commensal microbes. Bacterial counts were determined by plating serial dilutions of small intestine homogenates and facal suspensions on plate count agar (Difco), LB agar, and Eosin Methylene Blue (EMB) agar plates. Photographs of the facal suspension on plates were acquired with a Leica DFC 425C camera. The resultant fusion construct was chromosomally integrated in the resulting fusion construct was performed as previously described.

DNA purification from mouse faeces, small intestine and colon. Fresh stool pellets were collected before mice were killed. Faecal samples were stored at −80°C before DNA purification. Immediately after the mice were euthanized, their small intestines and colons were recovered manually. Total genomic DNA was extracted from the faecal samples using a QIAamp DNA Stool Mini Kit (Qiagen) according to the manufacturer’s protocol. DNA was precipitated with ethanol and resuspended in 50 μl of TE buffer with 100 μg ml−1 phenol. Total genomic DNA was extracted from intestinal samples using the QIAamp DNA Stool Mini Kit (Qiagen) with a minor modification. Briefly, the recovered mouse homogenates were homogenized in 2 ml of PBS and centrifuged at low speed to remove tissue debris. The resultant supernatants were subjected to the purification procedures outlined in the QIAamp DNA Stool Mini Kit.

16S rRNA gene quantification by qRT-PCR. The relative abundance of each specific bacterial group was determined by quantitative real-time PCR with reverse transcription (qRT–PCR). Two micro-litres of 10-fold diluted genomic DNA samples obtained from mouse faeces, small intestines, or colons served as the templates for SYBR Green-based quantitative PCR with reverse transcription (qRT–PCR) reactions. qRT–PCR was performed using a SYBR Premix Ex Taq kit (Takara Bio Inc., Japan) and gene-specific primers that were designed to amplify the 16S region as previously described. The relative abundance of each specific bacterial group was analysed by quantitative real-time PCR. Two micro-litres of faecal samples or 40 cycles (small intestine or colon samples) of 94°C for 30 s, 60 or 62°C for 45 s, and 72°C for 1 min. The level of each gene was normalized to that of the housekeeping gene gapdh. Results are expressed relative to the 16S rRNA level obtained with species-specific primers from an intestinal sample isolated from control mice.

Catalase activity assays. A neutral gel-based catalase activity assay was performed as previously reported. Briefly, proteins in bacterial lysates were resolved on a 7.5% nondenaturing acrylamide gel and stained with 3,3′-diaminobenzidine (DAB) (Sigma). Lysates were prepared by sonicating bacterial cell suspensions in 50 mM Tris-HCl, pH 7.4.

Catalase activity assays. The eKatE gene and its own promoter was PCR amplified and cloned into the multicloning site of pBAD24 plasmid. The resultant plasmid, named pBAD24: eKatE, was transformed into the E. coli strain. PCRamers for cloning were ligated into pBAD24: eKatE plasmids and transformed into E. coli DH5α (KanR) by electroporation. After the transformants were selected by blue/white screening, the plasmids were purified and sequenced by BLASTp analyses of catalase proteins. To provide further information about the distribution of catalase proteins among commensal microbes in the human gut, we BLAST searched amino-acid sequences obtained from an integrated catalogue of

Whole-genome sequence and annotation. The Ec and atEc strains were grown in LB medium at 37°C for 15–16 h, with shaking under aerobic conditions. Genomic DNA was extracted from bacteria using the G-Spin Genomic DNA Extraction Kit (NIPON Biotechnology Inc.). Bacterial genomes were sequenced on an Illumina MiSeq platform (Illumina, Inc., USA). Sequence reads (tec: 7,156,532 reads with a total read length of 2,281,359,270 bp; atEc: 6,496,870 reads with a total read length of 1,721,860,260 bp) were assembled de novo using GS Assembler v. 2.6 (Roche Diagnostics) and GCL genomics workbench 6.0 (GCL bio, Denmark). This assembly resulted in 62 and 152 contigs for teC and atEc, respectively. The contigs and PCR-based long reads were combined through manual annealing and used to produce the genome assemblies.

Bioinformatic analyses. Tetranucleotide frequency and CAI were calculated following the procedures described elsewhere. The pairwise overall similarity between genome sequences was calculated using the OrthoANI method and the dendrogram (shown in Supplementary Fig. 5) was generated using the UPGMA method. Sequences for phylogenetic analysis of KatG and KatE were retrieved from the GenBank database using the BLAST algorithm. Sequences were aligned with MAFFT v7.100 (ref. 77) and aligned positions with > 50% gaps were removed using GBLOCKS v0.91 (ref. 63). The phylogenetic relationships were inferred with RAxML v8.2.4 (ref. 64) and the trees (shown in Supplementary Fig. 7) were visualized using Dendroscope v3.2.2 (ref. 65). The trees were rooted by proteins that belong to the basal clade of each catalase group.
reference genes in the human gut microbiome. This comprehensive catalogue is composed of 9,879,896 non-redundant genes, which is a near complete set of genes found in almost all human gut bacteria. We also searched against an integrated gene catalogue of the mouse gut metagenome to outline the relative abundance of catalase proteins in human versus mouse intestine. The mouse metagenome catalogue comprises 2,572,074 non-redundant bacterial genes present in faecal samples collected from 184 mice of diverse backgrounds. We downloaded the amino-acid sequences listed in the companion web-servers and used those for pairwise sequence alignments using the BLASTp algorithm.

Statistical analysis. Data are expressed as mean ± standard error of the mean (SEM). Unpaired Student’s t-test and ANOVA (for Fig. 4d and Supplementary Fig. 1d-f) were used to determine whether differences between groups were significant. A P value <0.05 was considered to indicate statistical significance. All experiments were repeated for reproducibility.

Data availability. The genomic sequences of the t3c and at3c strains have been deposited in the NCBI genome database with accession codes LRA00000000 and LRBX00000000, respectively. The authors declare that all other data supporting the findings of this study are available within the article and its Supplementary Information files.

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**Author contributions**

M.Y.Y. and S.S.Y. conceived, designed and coordinated the study. M.Y.Y., K.B.M., K.M.L, Y.Y.K., Y.T.O and K.L. performed the experiment and acquired the data. J.C., B.Y.K., S.H.Y., I.L. and C.Y.K. performed bioinformatic analyses. S.S.Y. and M.Y.Y. wrote the paper. All the authors participated in discussions of the results and reviewed the final draft.

**Additional information**

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