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ORIGINAL ARTICLE

Lactobacillus reuteri and *Escherichia coli* in the human gut microbiota may predict weight gain associated with vancomycin treatment

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BACKGROUND: Antibiotics, used for 60 years to promote weight gain in animals, have been linked to obesity in adults and in children when administered during early infancy. *Lactobacillus reuteri* has been linked to obesity and weight gain in children affected with Kwashiorkor using ready-to-use therapeutic food. In contrast, *Escherichia coli* has been linked with the absence of obesity. Both of these bacteria are resistant to vancomycin.
OBJECTIVES AND METHODS: We assessed vancomycin-associated weight and gut microbiota changes, and tested whether bacterial species previously linked with body mass index (BMI) predict weight gain at 1 year. All endocarditis patients treated with vancomycin or amoxicillin in our center were included from January 2008 to December 2010. *Bacteroidetes, Firmicutes, Lactobacillus* and *Methanobrevibacter smithii* were quantified using real-time PCR on samples obtained during the 4–6 weeks antibiotic regimen. *L. reuteri, L. plantarum, L. rhamnosus, Bifidobacterium animalis* and *E. coli* were quantified on stool samples obtained during the first week of antibiotics.
RESULTS: Of the193 patients included in the study, 102 were treated with vancomycin and 91 with amoxicillin. Vancomycin was associated with a 10% BMI increase (odds ratio (OR) 14.1; 95% confidence interval (CI; 1.03–194); $P = 0.047$) and acquired obesity (4/41 versus 0/56, $P = 0.01$). In patients treated with vancomycin, *Firmicutes, Bacteroidetes* and *Lactobacillus* increased, whereas *M. smithii* decreased ($P < 0.05$). The absence of *E. coli* was an independent predictor of weight gain (OR = 10.7; 95% CI (1.4–82.0); $P = 0.02$). Strikingly, a patient with an 18% BMI increase showed a dramatic increase of *L. reuteri* but no increase of *E. coli*.
CONCLUSION: The acquired obesity observed in patients treated with vancomycin may be related to a modulation of the gut microbiota rather than a direct antibiotic effect. *L. reuteri*, which is resistant to vancomycin and produces broad bacteriocins, may have an instrumental role in this effect.

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Keywords: antibiotics; obesity; gut microbiota; *Lactobacillus*; *Escherichia coli*

INTRODUCTION

Stokstad *et al.*[1] first found that the administration of *Streptomyces aureofaciens* led to a twofold weight increase in chickens, and identified that one of its bacteriocins, chlortetracycline, was responsible for this effect. For over 60 years since then, antibiotics, including mainly glycopeptides, tetracycline, macrolides and penicillins, have been used at subtherapeutic levels to promote weight gain in animals. These continue to be widely used in the United States of America, where more than 13 000 tons of active ingredients have been sold in 2009.[2] The Animal Health Institute of America (www.ahi.org) has estimated that without the use of growth promoting antibiotics, the USA would require an additional 452 million chickens, 23 million cattle and 12 million pigs to reach the levels of production attained with current practices.[3] From the beginning of their use in agriculture in the 1950s, a similar effect on growth was reported in humans,[4–6] but seems to have been neglected. The link recently identified between the gut microbiota, human obesity[7] and malnutrition[8,9]

has led researchers to reconsider the impact of antibiotics that are associated with obesity particularly when administered in early infancy (Table 1).

Glycopeptides, derived from bacteriocins secreted by bacteria of the *Actinomycetales* order, are antibiotics largely used in human medicine but linked with weight gain both in animals and humans. Indeed, avoparcin, originally isolated from *Streptomyces candidus*, was associated with weight gain in farm animals,[10] whereas vancomycin, which is isolated from *Amycolatopsis orientalis*, was associated with significant weight gain and acquired obesity in humans[11] in addition to increased adiposity in animals.[12] *In vitro*, glycopeptides affect mainly Gram-positive bacteria due to inhibitory activity against peptidoglycan synthesis. However, *Pediococcous* and *Lactobacillus* are not susceptible to glycopeptides, with the notable exception of the *L. acidophilus* group. Even if the susceptibility depends on the bacterial species within the same genus, treatment with oral vancomycin in humans has been associated with a decrease in *Clostridium*,

Enterococcus, Staphylococcus, Bacteroides and Bifidobacterium. However, an overgrowth of Lactobacillus, Pediococcus and Proteobacteria as Klebsiella, Enterobacter or Citrobacter has also been reported (Table 2).

In a previous study, we found an association between vancomycin and acquired obesity in humans, but gut microbiota was not analyzed.[11] In this work, we have tested the weight gain effect associated with vancomycin in a new series of patients. In addition, we analyzed the change in the gut microbiota and tested whether bacterial species that were previously linked with body mass index (BMI)[13,14] were predictive of weight gain after vancomycin treatment.

Table 1. Studies reporting a significant weight gain associated with antibiotic administration in humans

Antibiotics	Indication	References
All	Infections in the early life	22,23
Glycopeptides		
Vancomycin	Endocarditis	11
Betalactamines		
Amoxicillin	Malnutrition	8,32
Ceftriaxone	Malnutrition	32
Cefdinir	Malnutrition	8
Macrolides		
Azithromycin	Cystic fibrosis	37–39
Erythromycin	Neonatology	40
Clarithromycin	Eradication of *Helicobacter pylori*	41,42
Tetracyclines		
Minocyclin	Cystic fibrosis	43
Chlortetracycline	Neonatology	6
Chlortetracycline	Antibiotic prophylaxis on immune response	4

PATIENTS AND METHODS

Patients

All endocarditis patients who were treated by vancomycin or amoxicillin (taken as a control group based on results of our previous study[11]) in the departments of Cardiology, Hospital de la Timone, Marseille, France were included (Figure 1) between January 2008 and December 2010. Endocarditis was defined using the modified Duke criteria.[15] Patients were treated for at least 4 weeks with or without gentamycin. For each case of endocarditis, the decision for surgery was made by a multidisciplinary discussion following our protocol.[16] The use of any antibiotic other than amoxicillin, vancomycin or gentamycin for more than 7 days and switching between vancomycin and amoxicillin were exclusion criteria. As all the patients were included after the diagnosis of endocarditis, samples were obtained from the first week after the start of antibiotics. The control group included 42 controls whose stools were analyzed in a previous study.[14] Collection of data included age, sex, height, weight and type of antibiotic treatment, but other medications or diet were not collected. According to our published protocols,[16] most of patients (>80%) received 6 weeks of treatment. Written consent was obtained from each participant and approved by the ethics committee of the Faculty of Medicine La Timone, Marseille, France under number 08–002.

Analysis of weight change

A standardized questionnaire was used to collect demographical, clinical and therapeutic data in all patients treated by antibiotics. The baseline weight (1 month before the onset of the disease), weight at 1 year and height were collected for each patient based on clinical records, systematic follow-up consultations or phone calls. The %deltaBMI, a %deltaBMI >10% and an acquired obesity (BMI becoming >30 at 1 year) were assessed comparing baseline BMI and BMI at 1 year. The %deltaBMI was calculated as follows: %deltaBMI = (BMI at 1 year − baseline BMI)/baseline BMI × 100.

Analysis of intestinal flora

Starting in April 2009, all patients with an endocarditis diagnosis were ask to provide a stool sample at diagnosis and as much as possible during the 4–6 weeks of treatment. DNA was isolated from the stool as described in Dridi et al.[18] The purified DNA was eluted from samples to a final volume of 100 μl and stored at −80 °C until analysis. Real-time PCR analysis was performed on a Stratagene MX3000 (Agilent, Santa Clara, CA, USA) using the Quantitect PCR mix (Qiagen, Courtaboeuf, France) as previously described,[19] which targeted Bacteroidetes, Firmicutes, Lactobacillus and M. smithii. Quantification of all bacteria was performed as previously described.[18]

Table 2. Effect of vancomycin and amoxicillin on gut microbiota in the literature

	Host	Method	References
Vancomycin			
Overgrowth of lactobacilli[a] (and pediococci)	Humans	Cultivation	44–46
Increase of *Lactobacillaceae*[a]	Humans	Cloning	47
Increase of *Proteobacteria*[a]	Humans	Cloning	47
Decrease of Enterococci	Humans	Cultivation	44,45
Decrease of Staphylococci	Humans	Cultivation	46
Strong suppression or elimination of *Bacteroides*[b]	Humans	Cultivation	44,45
Decrease of clostridia and bifidobacteria[b]	Humans	Cultivation	45
Decrease of *C. leptum, C. coccoides, C. symbosium*	Mouse	PCR-DGGE	48
Amoxicillin			
Increase of aerobic Gram-negative rods such as enterobacteria other than *E. coli* (Klebsiella, Enterobacter)	Humans	Cultivation	49–52
Increase of Gram-negative bacilli	NCS mice	Cultivation	36
Decrease of streptococci and staphylococci	Humans	Cultivation	50
Almost suppression of lactobacilli[a]	Rat pups	Cultivation	53
	NCS mice	Cultivation	36
Depletion of enterococci	Rat pups	Cultivation	53
Enterococci increase after transient depletion	NCS mice	Cultivation	36
Depletion of enterobacteriacae[b]	Rat pups	Cultivation	53

Abbreviations: BMI, body mass index; DGGE, denaturing gradient gel electrophoresis. [a]Specific lactobacilli (members of the *Lactobacillaceae* family) have been associated with obesity,[14] correlated with BMI[13] and associated with weight gain.[27] [b]Specific *Proteobacteria*, enterobacteriaceae and *E. coli* have been linked with both weight gain and obesity or weight loss or absence of obesity, whereas *Bacteroidetes*, *Bacteroides* and bifidobacteria have been linked to a statistical anti-obesity effect.[54]
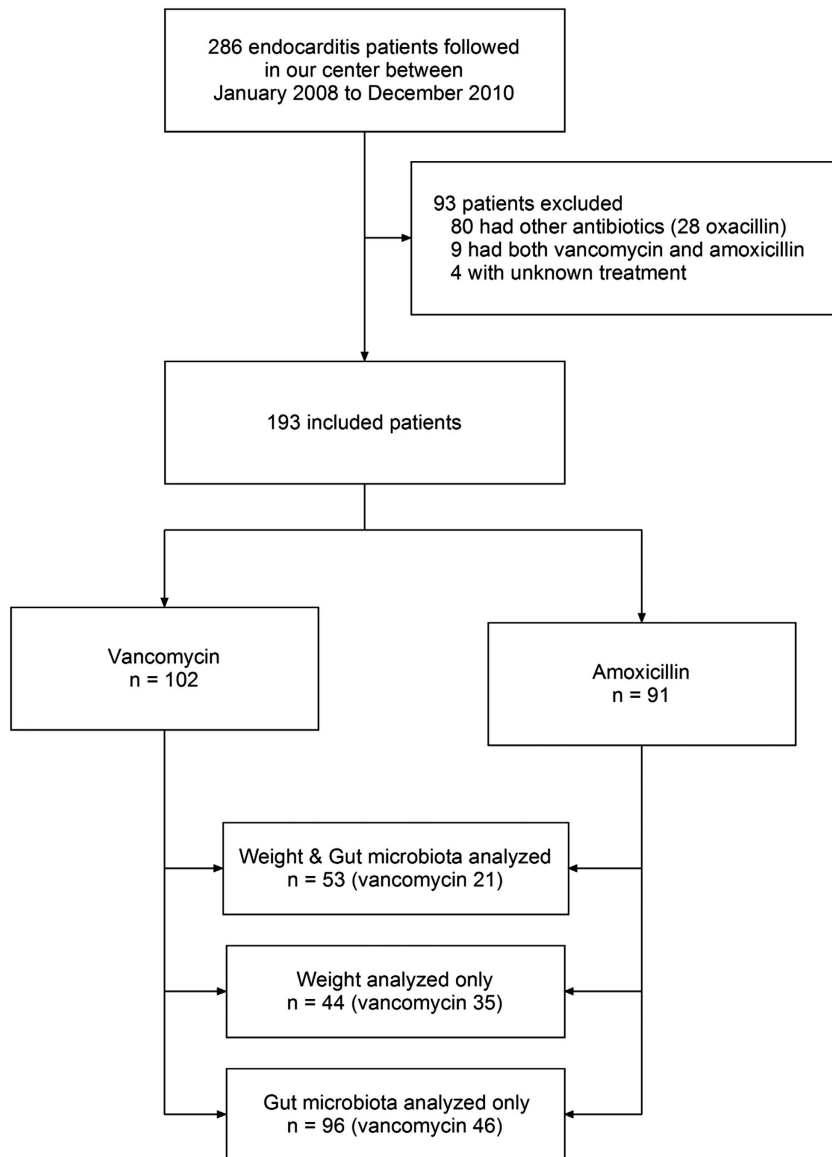
Figure 1. Study flowchart.

A second analysis designed for the identification of gut bacteria predictive of weight gain was performed only for samples obtained during the first week of antibiotics in patients for whom both weight and gut flora data were available. A real-time PCR was performed on a Bio-Rad FLX96 and targeted *L. reuteri*, *L. plantarum*, *L. rhamnosus*, *B. animalis* and *E. coli* as previously reported.[13]

Statistical analysis

Student's *t*-test or a Mann–Whitney test was used to compare continuous variables between the two groups according to the distribution assessed by the Kolmogorov–Smirnov test. Proportions were compared using a two-sided Barnard's exact test.[20] The analyses were performed using SPSS v21.0 (IBM, Paris, France).

In the first analysis, the changes of BMI at 1 year were compared between the two antibiotic groups (vancomycin or amoxicillin) for all patients with available information. A logistic regression analysis was performed, using a deltaBMI > 10% or acquired obesity as the dependent variable. The regression was adjusted by age, sex, surgery and baseline BMI, and was systematically adjusted by antibiotic treatment (vancomycin or amoxicillin).

In the second analysis, the concentrations of *Bacteroidetes*, *Firmicutes*, *M. smithii* and *Lactobacillus* were compared between all samples by intervention group (vancomycin or amoxicillin) and controls for all patients

with an available stool sample. In a preliminary univariate analysis, all significant comparisons were confirmed using Dunn's multiple comparison test. To account for the intra-individual correlation within the repeated measures (one individual could have several fecal samples), a linear model with mixed effects and a random intercept was used, adjusted for age, sex and baseline BMI.

Finally, to identify a relationship between the initial gut microbiota and weight gain at 1 year, proportions of endocarditis patients with weight gain were compared between carriers and non-carriers for each bacterium analyzed on samples obtained during the first week of antibiotics. When comparing %deltaBMI at 1 year between carriers and non-carriers, a bilateral Student's *t*-test was performed with Welch's correction after the Kolmogorov–Smirnov test.

RESULTS

Weight change study

Ninety-seven patients were analyzed for weight change at 1 year (Figure 1). The proportion of patients with an increase for more than 10% BMI was higher in the vancomycin group (5/41 (12.2%)) than in the amoxicillin group (1/56 (1.8%), $P = 0.038$). This finding was confirmed using a logistic regression analysis, adjusted for age, sex, surgery and baseline BMI (Tables 3 and 4). It was not

4

significant for an increase in BMI >5% (9/41 in the vancomycin group versus 7/56 in the amoxicillin group, $P = 0.23$). Acquired obesity was observed in four individuals who had all been treated with vancomycin ($P = 0.01$). One of the individuals with acquired obesity was initially lean (BMI 25) and gained 11 kg, whereas the other three have baseline BMI between 27 and 29.

Gut microbiota alteration during antibiotic treatment

One-hundred and forty-nine patients were analyzed for gut microbiota alteration during antibiotic treatment (Figure 1). The amount of *Firmicutes* was significantly higher in the vancomycin

Table 3. Baseline characteristics of patients from the weight change study

	Amoxicillin (n = 56)	Vancomycin (n = 41)	P-value (test)
Sex (Male)	46/56	28/41	0.18 (Fisher)
Age	62.5 ± 11.1	65.3 ± 12.8	0.31 (Student)
Surgery[a]	36/56	18/41	0.07 (Fisher)
Baseline BMI	26.8 ± 5.1	26.2 ± 4.8	0.53 (Student)

Abbreviation: BMI, body mass index. [a]Data unavailable for six patients. Two-sided tests. Most females were postmenopauzal so that estrogen driven gender differences in gut microbiota do not seem relevant in our study.[55]

Table 4. Logistic regression of a BMI increase over 10% at 1 year according to antibiotics, age, sex, surgery and baseline BMI

	OR (95% CI), P-value
Vancomycin	14.1 (1.03–194.1), $P = 0.047$
Age	0.92 (0.86–0.995), $P = 0.037$
Male sex	0.11 (0.016–0.87), $P = 0.036$

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

group when compared with controls ($P = 0.007$), whereas the amoxicillin group showed no significant difference compared with the control group ($P = 0.58$) (Figure 2). The amount of *Bacteroidetes* was also increased in the vancomycin group when compared with controls ($P < 0.0001$). Similarly, the amount of *Bacteroidetes* was higher in the amoxicillin group ($P = 0.002$). Conversely, despite *in vitro* resistance to the use of antibiotics,[21] the amount of *M. smithii* was significantly decreased in both groups of patients on antibiotics when compared with controls ($P = 0.01$ for the vancomycin group and $P = 0.01$ for the amoxicillin group). In addition, there was no significant difference in the amount of *M. smithii* between patients receiving vancomycin and amoxicillin ($P = 0.80$). *Lactobacillus* was significantly increased in the vancomycin group compared with controls ($P = 0.04$). There was no significant difference in the amount of *Lactobacillus* between the amoxicillin group and the control group. Finally, the total number of bacteria was increased in both groups of patients receiving antibiotics when compared with controls ($P < 0.0001$ and $P = 0.001$ for vancomycin and amoxicillin, respectively). No significant results were obtained in the multivariate analysis.

Case of a patient with acquired obesity while being initially lean

A 51-year-old woman treated with vancomycin after a valve replacement surgery presented a massive weight gain over the course of a year (height: 1.50 m; weight: 58 kg before endocarditis increasing to 69 kg at 1 year; BMI: 25.8 before the onset of the disease increasing to 30.6, 1 year after the treatment corresponding to an increase of 11 kg, 4.8 kg m^{-2} and 19% BMI). The analysis of her first stool sample collected promptly after the beginning of the vancomycin treatment was devoid of *L. reuteri*, *L. plantarum*, *L. rhamnosus*, *B. animalis* and *E. coli*. *L. reuteri* was found in high concentrations (4.7 log10 copies DNA per ml) for the first time in her second stool sample, which was collected 68 days after beginning the vancomycin treatment, no *E. coli* was detected and there was a decrease in *Firmicutes* (7.9–7.0 log c.f.u. per ml), *Bacteroidetes* (8.6–7.9 log c.f.u. per ml) and *M. smithii* (5.8–4.8 log c.f.u. per ml) between the two samples.
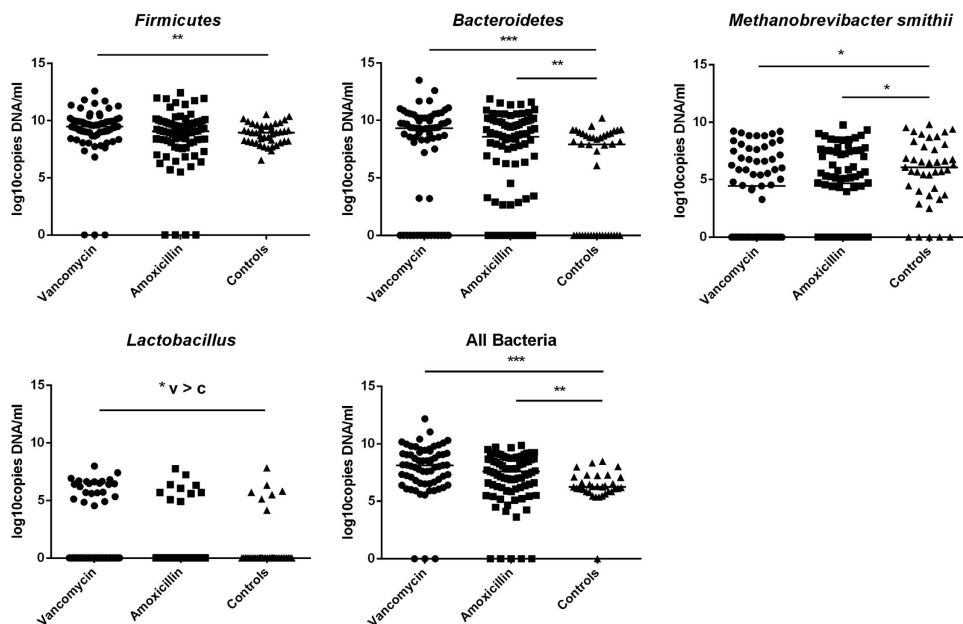

Figure 2. Global modification of the gut microbiota during long-term amoxicillin or vancomycin treatment. *$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$ compared with controls. All the significant comparisons have been confirmed after Dunn's multiple comparisons test.

Absence of *E. coli* is predictive of weight gain under vancomycin

In the 21 patients treated with vancomycin for whom a stool sample obtained during the first week of antibiotics was available, the absence of *E. coli* in the initial gut microbiota was associated with an increase in BMI at 1 year (8/10 with a weight gain versus 3/11 without weight gain, $P = 0.03$). A logistic regression model adjusted for age, sex and surgery identified an association between the absence of *E. coli* and weight gain at 1 year (odds ratio = 10.7; 95% confidence interval 1.39–82.0); $P = 0.02$). This was confirmed using %deltaBMI (mean ± standard Error, 4.0 ± 2.1 for patients without *E. coli* versus − 3.4 ± 2.0 for patients with *E. coli*, $P = 0.01$, Figure 3). This was also true when one outlier (a patient with a BMI increase of 19%) was excluded ($n = 20$, 2.5 ± 1.6 versus − 3.4 ± 2.0, $P = 0.03$). We did not find any other significant differences regarding other bacteria (*L. plantarum*, *L. reuteri*, *L. rhamnosus*, *B. animalis*, Firmicutes, Bacteroidetes, *M. smithii* and the *Lactobacillus* genus) or data from the amoxicillin-treated patients. However, the prevalence was extremely low for *Lactobacillus* and *Bifidobacterium*, as previously reported.[13]

DISCUSSION

In this study, we confirmed that vancomycin was associated with a massive weight gain compared with the weight before the onset of the diseases (endocarditis) in 1/8 human individuals, whereas amoxicillin caused such a massive weight gain in <1 in 50 individuals. We found that the initial gut microbiota was a critical parameter for the identification of individuals more likely to gain weight under vancomycin. Indeed, the presence of *E. coli* at the beginning of treatment was predictive of weight loss, whereas its absence was predictive of weight gain. In contrast, the
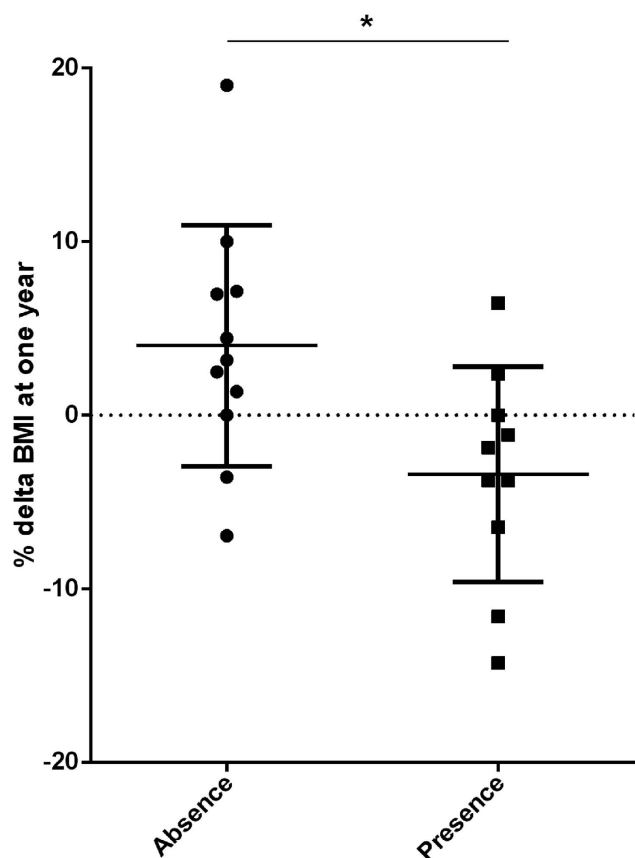

Figure 3. Weight change at 1 year in patients under vancomycin treatment according to the presence or absence of *E. coli* in the initial gut microbiota. *$P < 0.05$.

only patient with an acquired obesity while being initially lean showed a dramatic increase in *L. reuteri*. Finally, we confirmed a characteristic profile of the vancomycin gut microbiota with increased levels of *Lactobacillus*, increased Firmicutes and Bacteroidetes, but decreased levels of *M. smithii*.

Gut microbiota alterations associated with vancomycin have been previously reported using culture or large-scale molecular studies and included mainly an increase in the Proteobacteria and Lactobacillaceae, and a specific decrease in specific Firmicutes representatives, such as *Clostridium*, *Staphylococcus* and *Enterococcus*. One of the most striking difference between vancomycin and amoxicillin, the latter used in our study as a control, is the increase in *Lactobacillus* associated with vancomycin (Table 2).

Human obesity, which is promoted by antibiotics when administered in early infancy,[22,23] was associated with a specific profile of the digestive microbiota and a causal role was strongly suggested by the microbiota transplantation experiments.[24] *Clostridium perfringens*,[21,25,26] *C. difficile*,[21] *C. tetani*,[21] *C. botulinum*,[21] *E. coli*[13] and *M. smithii*[13,14,17] have been linked with the absence of obesity. Conversely, *Lactobacillus* and especially *L. reuteri* or *L. sakei* have been linked with obesity.[13,14,19,27,28] A very recent study[21] comparing diabetic and nondiabetic women confirms the key role of *Lactobacillus* in weight regulation in humans as four species, *L. fermentum*, *L. gasseri*, *L. johnsonii* and *L. crispatus* were positively correlated with BMI, whereas a single species, *L. casei* was negatively correlated with BMI.[21] Consistently, we had previously shown that *L. fermentum* was associated with weight gain,[27] whereas *L. casei* or *L. plantarum* were associated with the absence of obesity.[14,27] In one study on mice, *L. reuteri* L6798 was associated with weight gain, whereas *L. reuteri* ATCC PTA 4659 was associated with weight loss.[29] These results suggest a species and strain specificity in the effect of *Lactobacillus* probiotics on weight regulation.[30] Overall, *Lactobacillus* provided a biological plausibility for the link between vancomycin and weight gain.

The acquired obesity associated with a dramatic *L. reuteri* increase, whereas treated by vancomycin in one patient suggest that bacteriocins could be critical in the link between weight and the profile of the gut microbiota. Indeed, *Lactobacillus* and particularly *L. reuteri* produce broad-spectrum bacteriocins, which allow for the elimination of various enteropathogens as *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Salmonella* and *E. coli*, the latter being associated previously to the absence of obesity.[31] In addition, the weight increase in children with Kwashiorkor under treatment with ready-to-use therapeutic food (RUTF) was correlated with an increase of *L. reuteri* and *L. gasseri*, and considered to be associated with bacteriocins secretion, resulting in a dramatic gut microbiota alteration.[9] Consequently, the effectiveness of antibiotic treatment to combat severe malnutrition[8,32] also supports the role of bacteriocins, as they could increase the ability of the gut microbiota to extract energy from the diet, as found in experimental models.[12]

Our study includes some limitations. First, all patients had parenteral antibiotic administration. The administration routes have been shown to impact significantly the shift on the gut microbial populations with oral administration being associated with more pronounced alteration of the gut microbiota,[33] but intravenous administration was also associated with dramatic gut microbiota changes notably in infants.[34] As a confirmation, we found in this study using intravenous route a reproducible alteration previously obtained by oral administration as a *Lactobacillus* increase under vancomycin. In addition, although large-scale molecular methods became recently accurate at the species level,[21] studies targeting bacterial species of interest in the human gut microbiome as in our work remain completely relevant.[13,14] As such, species-specific PCR avoids some biases of large-scale molecular methods such as the primer bias.[35]

In conclusion, the major weight gain and acquired obesity specifically observed in some patients treated with vancomycin could be related to the selection of resistant bacteria previously associated with obesity, such as an increase in *Lactobacillus*, and/or to the suppression of bacteria that are associated with the absence of obesity, such as *C. perfringens*,[21,25,26] *C. difficile*,[21] *C. tetani*,[21] *C. botulinum*,[21] *E. coli*[13] or *M. smithii*.[13,14,17] Bacteriocins, typically secreted by *Lactobacillus*,[31] which are selected by vancomycin, may explain why the effect of an antibiotic on a complex microbial population is much wider than the expected direct 'in vitro' effect, by killing populations paradoxically resistant to the antibiotic when used 'in vivo'. As demonstrated in experimental studies,[36] we demonstrated that weight gain effect following antibiotic administration depends both on the initial gastrointestinal microbiota and the antibiotic used. From our results, it seems necessary to inform the patient of the risk of weight gain and obesity during long-term antibiotic treatment, especially when the patient is treated with vancomycin. More generally, this work supports the instrumental role of the human gut microbiota in the pro-obesity effect of antibiotics as recently reported,[22,23] and suggests bacteriocins as a keystone between gut microbiota and obesity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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