SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
BACTERIAL TECHNIQUES AND APPLICATIONS
PATHOGENS

Received
17 July 2014

Accepted
31 October 2014

Published
24 November 2014

Correspondence and requests for materials should be addressed to F.B. (fadi.bittar@univ-amu.fr) or D.R. (didier.raoult@gmail.com)

Gorilla gorilla gorilla gut: a potential reservoir of pathogenic bacteria as revealed using culturomics and molecular tools

Fadi Bittar[1], Mamadou B. Keita[1], Jean-Christophe Lagier[1], Martine Peeters[2], Eric Delaporte[2] & Didier Raoult[1]

[1]Aix Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, Inserm 1095, 13005 Marseille, France, [2]Institut de Recherche pour le Développement, University Montpellier 1, UMI 233, Montpellier, France.

Wild apes are considered to be the most serious reservoir and source of zoonoses. However, little data are available about the gut microbiota and pathogenic bacteria in gorillas. For this propose, a total of 48 fecal samples obtained from 21 *Gorilla gorilla gorilla* individuals (as revealed via microsatellite analysis) were screened for human bacterial pathogens using culturomics and molecular techniques. By applying culturomics to one index gorilla and using specific media supplemented by plants, we tested 12,800 colonies and identified 147 different bacterial species, including 5 new species. Many opportunistic pathogens were isolated, including 8 frequently associated with human diseases; *Mycobacterium bolletii, Proteus mirabilis, Acinetobacter baumannii, Klebsiella pneumoniae, Serratia marcescens, Escherichia coli, Staphylococcus aureus* and *Clostridium botulinum*. The genus *Treponema* accounted for 27.4% of the total reads identified at the genus level via 454 pyrosequencing. Using specific real-time PCR on 48 gorilla fecal samples, in addition to classical human pathogens, we also observed the fastidious bacteria *Bartonella* spp. *Borrelia* spp., *Coxiella burnetii* and *Tropheryma whipplei* in the gorilla population. We estimated that the prevalence of these pathogens vary between 4.76% and 85.7%. Therefore, gorillas share many bacterial pathogens with humans suggesting that they could be a reservoir for their emergence.

The African great apes, including gorillas, are a reservoir and source of human pathogens[1–6]. Calvignac-Spencer et al. identified 16 viral genera from wild great apes, including 8 genera that could be transmitted between humans and apes[7]. Among these different transmissible viruses, the Ebola and Marburg viruses are the most virulent pathogens and have caused multiple human outbreaks due to direct handling of gorillas and chimpanzees[2,8]. Furthermore, there is strong evidence that the human immunodeficiency virus originated from simian immunodeficiency viruses in chimpanzees and gorillas[4]. In addition to viruses, parasites, including *Plasmodium falciparum* and *Plasmodium vivax*, nematodes (such as *Trichuris*, *Ascaris*, *Oesophagostomum*, *Strongyloides*, and *Trichostrongylus*) and the Anoplocephalid cestode can infect both humans and great apes[3,9].

However, there are limited data on pathogenic bacteria in the gorilla. These bacteria include *Staphylococcus aureus*, *Escherichia coli*, *Rickettsia felis*, *Bacillus anthracis*-like bacteria, *Salmonella* spp., *Campylobacter* spp. and *Shigella* spp.[9–12]. Additionally, *Mycobacterium tuberculosis* and leprosy caused by *Mycobacterium leprae* have been observed in nonhuman primates[13,14]. Furthermore, Rwego et al. demonstrated the possibility of gastrointestinal bacterial transmission between humans and gorillas sharing the same habitat[15]. Therefore, studying the composition of bacterial communities in the gorilla gastrointestinal tract is important.

Although pyrosequencing is a rapid and efficient method for determining the bacterial phyla, it is limited, especially for bacterial identification at the genus and species levels[16].

Microbial culturomics (large-scale culture conditions followed by mass spectrometry or 16S rRNA identification of the isolated colonies) was proved to be an efficient method to explore the gut microbiota not only because it is able to isolate a high number of bacterial species including new species but also because it is more sensitive than metagenomic methods to detect minority populations including pathogens[16–18].

In this study, we explored the prevalence of human bacterial pathogens in wild gorillas from southern Cameroon. We exhaustively analyzed one stool sample using culturomics and pyrosequencing to detect the

commensal bacteria that are potential human pathogens[19]. Forty-eight additional fecal samples from wild gorillas were screened via real-time PCR to examine the prevalence of the human bacterial pathogens that were identified in the first stool sample using culturomics and of other human pathogens, including fastidious bacteria that are tested routinely in our lab for the diagnosis of infections in Africa.

Results

Source of fecal samples, microsatellite analyses and genetic identification of gorilla individuals. Fecal samples from western lowland gorillas (*Gorilla gorilla gorilla*) were collected at two sites in south-central Cameroon: near the Minton village (this sample was selected and used for exhaustive examination using culturomics, pyrosequencing and real-time PCRs because of its enough volume required for these analyses), and 47 fecal samples were collected near the Messok village (these samples were used for the molecular screening of human bacterial pathogens via real-time PCRs). A microsatellite analysis of 48 fecal samples enabled the identification of 21 gorilla individuals: 19 males and 2 females (Supplementary Table 1).

Bacterial diversity of gorilla gut using culturomics. In this study, a total of 86 culture conditions were tested: the optimal conditions applied for human gut exploration (Supplementary Table 2)[16] and innovative media, which were developed from plants (Supplementary Table 2). From one fecal sample, a total of 12,800 colonies were tested and 147 bacterial species were observed (Table 1 and Supplementary Table 2). The bacteria belonged to 47 genera divided into 4 phyla; Firmicutes (62.6%), Actinobacteria (24.5%), Proteobacteria (12.2%) and Fusobacteria (0.7%) (Table 1 and Supplementary Table 2). A comparative analysis showed that 113/147 (76.9%) of the bacterial species observed in the gorilla gut using culturomics were also observed in the human gut using culturomics (Table 1). Among the 86 different culture conditions tested, the most effective was a culture vial supplemented with rumen fluid in an anaerobic atmosphere followed by incubation for 11 days; this isolated 19 bacterial species (Supplementary Table 2). The incubation of the stool sample in aerobic or anaerobic enriched culture vials yielded 60 bacterial species (40.8%) including 50 (34%) anaerobic species (Supplementary Table 2).

Bacterial analyses of gorilla gut using specific plant media. Because gorillas are principally herbivores and because the gut microbial diversity may be influenced by diet, we designed novel media using tobacco leaves and tropical plants including the mango, papaya and banana fruits (see Methods and Supplementary Table 2). Of the 147 bacterial species observed, 60 (40.8%) grew on media supplemented with plant extracts, including 28 strains (19.04%) that were isolated exclusively using these media (Supplementary Table 2). The best plant condition was a culture vial supplemented with banana in an anaerobic atmosphere, used here for the first time, which enabled the isolation of 17 bacterial species (Supplementary Table 2).

New bacterial species and human pathogens in the gorilla gut. In addition to previously known bacteria (Table 1 and Supplementary Table 2), five new species were isolated in the fecal sample analyzed via culturomics, and their genomes were sequenced, including one new genus, *Gorillibacterium massiliense*[20], and 4 new species: *Bacillus massiliogorillae*[21], *Microbacterium gorillae*, *Paenibacillus gorillae*[22] and *Paenibacillus camerounensis* (Table 1 and Supplementary Table 2). The latter two species were recovered only from media supplemented with mango fruit (Supplementary Table 2). Notably, several potential human pathogens, including *Proteus mirabilis*, *Klebsiella pneumoniae*, *Mycobacterium bolletii*, *S. aureus*, *Clostridium botulinum*, *Acinetobacter baumannii*, *E. coli*, *Enterococcus faecalis*, *Enterococcus faecium*, *Clostridium perfringens* and *Serratia marcescens*, were isolated from this stool sample (Table 1 and Supplementary Table 2). *C. botulinum* was isolated twice using culture vials supplemented with rumen fluid and thioglycolate, and this species identification was performed by sequencing. *M. bolletii* was isolated using the MOD4 medium[23] and identified by sequencing the *rpoB* gene[24].

Bacterial diversity of gorilla gut via pyrosequencing. A total of 75,595 reads were obtained from pyrosequencing the fecal sample analyzed via culturomics. Of these, 36,463 reads with high-quality sequencing were analyzed and distributed into 11 phyla using the RDP classifier. Firmicutes composed the largest fraction (46.8% of the total reads), followed by Actinobacteria and Bacteroidetes (20% and 18.6%, respectively). Spirochaetes and Verrucomicrobia comprised 7.4% of the total reads (approximately 3.7% each). The remaining phyla, including Chloroflexi, Cyanobacteria, Fibrobacteres, Fusobacteria, Proteobacteria and Tenericutes, were represented by rare sequences. Finally, 4.6% of the reads were ascribed to unclassified bacterial phyla in the RDP database. Approximately 13% of the reads were identified at the genus level, yielding 38 genera. *Prevotella* (Bacteroidetes), *Treponema* (Spirochaetes) and *Bifidobacterium* (Actinobacteria) were the most abundant genera obtained from the 454 pyrosequencing (accounting for 40.8%, 27.4% and 11.3%, respectively, of the total reads identified at the genus level). Using the BLASTn tool in the NCBI website and setting the sequence similarity threshold to 98.7%, only 316 reads were identified at the species level, leading to the identification of 16 bacterial species.

Comparing the bacterial phyla found in the gorilla gut using culturomics and pyrosequencing with those of previous studies. Firmicutes followed by Actinobacteria comprised the majority of the detected bacteria in the fecal sample through both methods (i.e., culturomics and pyrosequencing). This is similar to the bacteria cultured from fecal samples from humans in Africa and Europe[16]. The high abundance of Firmicutes was reported previously in wild mountain gorillas[25], western lowland gorillas[26] and also in other primates including old world monkeys[27], chimpanzees[28] and wild pygmy loris[29]. Actinobacteria was the second most predominant phylum in our sample; this result is in agreement with the study by Vlckova et al. in captive western lowland gorillas[26]. Although Proteobacteria was the third most isolated phylum via culturomics, it was recovered from rare reads using pyrosequencing. Additional phyla such as Bacteroidetes, Cyanobacteria, Verrucomicrobia, and Tenericutes were identified in this fecal sample exclusively through pyrosequencing. All of these microbial divisions have been previously detected in human and nonhuman primates[16,26,27]. The remaining phyla found via metagenomics, including Spirochaetes, Chloroflexi and Fibrobacteres, are usually absent from the human gut[30] but are present in herbivore guts such as gorillas, colobuses (*Colobus guereza* and *Piliocolobus tephrosceles*) and guenons (*Cercopithecus ascanius*)[25,27].

Molecular detection of human pathogenic bacteria and estimation of their prevalence in the gorilla gut population tested. A total of 48 fecal samples from 21 gorillas were examined via specific real-time PCR to analyze the prevalence of pathogens identified using culturomics and other fastidious pathogens commonly tested in our center (Supplementary Table 3). Our molecular tests showed that the most prevalent bacteria in the gorilla gut were (in decreasing order of estimated prevalence) *K. pneumoniae* (80.95%), *Acinetobacter* spp. (76.19%), *Borrelia* spp. (47.6%), *S. aureus* (42.86%), *Bartonella* spp. (38%), *Pseudomonas aeruginosa* (38%), *S. marcescens* (33.33%), *Mycobacterium abscessus* (28.57%), *E. faecalis* (28.57%), *Coxiella burnetii* (23.8%), *E. coli* (19%), *A. baumannii* (19%) and *Tropheryma whipplei* (4.76%) (Table 2).

Table 1 | Bacterial species isolated via culturomics in the stool sample of gorilla and their previous description in the human gut and human diseases

Bacterial species	Bacterial species	Bacterial species	Bacterial species
*Acinetobacter baumannii**	*Brevibacterium epidermidis*†‡	*Gemella sanguinis*‡	*Pseudomonas stutzeri*†‡
Acinetobacter lwoffi	*Brevibacterium iodinum*‡	*Gorillibacterium massiliense*§	*Rhodococcus equi*†‡
Acinetobacter schindleri	*Brevibacterium linens*‡	*Granulicatella adiacens*†‡	*Rothia aeria*‡
Actinomyces naeslundii†‡	*Brevibacterium luteolum*	*Klebsiella pneumoniae**‡	*Rothia mucilaginosa*†‡
Actinomyces neuii†	*Brevibacterium permense*	*Kocuria polaris*	*Sarcina ventriculi*‡
Actinomyces odontolyticus†‡	*Brevundimonas aurantiaca*	*Kocuria rhizophila*‡	*Serratia marcescens**
Aerococcus viridans‡	*Clostridium baratii*†‡	*Kytococcus schroeteri*†‡	*Staphylococcus arlettae*‡
Agrococcus jenensis‡	*Clostridium bifermentans*†‡	*Kytococcus sedentarius*‡	*Staphylococcus aureus**‡
Arthrobacter castelli‡	*Clostridium botulinum**	*Lactobacillus paracasei*‡	*Staphylococcus capitis*†‡
Bacillus arsenicus‡	*Clostridium celerecrescens*	*Lactococcus garvieae*‡	*Staphylococcus caprae*†‡
Bacillus barbaricus	*Clostridium glycolicum*‡	*Lysinibacillus fusiformis*‡	*Staphylococcus cohnii*†‡
Bacillus bataviensis	*Clostridium lituseburense*‡	*Lysinibacillus sphaericus*‡	*Staphylococcus epidermidis*†‡
Bacillus cereus†‡	*Clostridium orbiscindens*‡	*Microbacterium flavum*	*Staphylococcus haemolyticus*†‡
Bacillus circulans†‡	*Clostridium paraperfringens*‡	*Microbacterium kitamiense*	*Staphylococcus hominis*†‡
Bacillus flexus‡	*Clostridium paraputrificum*‡	*Microbacterium gorillae*§	*Staphylococcus pasteuri*†‡
Bacillus humi	*Clostridium perfringens**‡	*Micrococcus luteus*‡	*Staphylococcus pettenkoferi*†‡
Bacillus idriensis	*Clostridium sordellii*†‡	*Micrococcus lylae*‡	*Staphylococcus saprophyticus*†‡
Bacillus licheniformis†‡	*Clostridium sporogenes*‡	*Moraxella osloensis*†‡	*Staphylococcus sciuri*‡
Bacillus massiliogorillae§	*Clostridium sporosphaeroides*	*Mycobacterium bolletii**	*Staphylococcus warneri*†‡
Bacillus massiliensis‡	*Clostridium symbiosum*‡	*Neisseria flavescens*‡	*Staphylococcus xylosus*†‡
Bacillus megaterium†‡	*Clostridium tertium*†‡	*Neisseria mucosa*‡	*Streptococcus alactolyticus*†‡
Bacillus mycoides‡	*Corynebacterium aurimucosum*†‡	*Neisseria perflava*‡	*Streptococcus cristatus*†‡
Bacillus niabiensis	*Corynebacterium minutissimum*†‡	*Paenibacillus azoreducens*	*Streptococcus gallolyticus*†‡
Bacillus novalis	*Corynebacterium pseudogenitalium*	*Paenibacillus barcinonensis*‡	*Streptococcus iniae*†
Bacillus oshimensis	*Corynebacterium variabile*	*Paenibacillus camerounensis*§	*Streptococcus mitis*†‡
Bacillus pumilus†‡	*Dietzia natronolimnaea*‡	*Paenibacillus glycanilyticus*‡	*Streptococcus oralis*†‡
Bacillus simplex‡	*Enterobacter cloacae**‡	*Paenibacillus illinoisensis*‡	*Streptococcus parasanguinis*†‡
Bacillus thuringiensis‡	*Enterococcus avium*†‡	*Paenibacillus gorillae*§	*Streptococcus peroris*†‡
Bacillus vireti	*Enterococcus casseliflavus*†‡	*Paenibacillus pabuli*	*Streptococcus salivarius*†‡
Bacillus weihenstephanensis	*Enterococcus faecalis**‡	*Paracoccus yeei*‡	*Streptococcus sanguinis*†‡
Bifidobacterium dentium†	*Enterococcus faecium**‡	*Propionibacterium acnes*†‡	*Streptomyces fungicidicus*
Brachybacterium paraconglomeratum	*Enterococcus mundtii*†	*Propionibacterium avidum*‡	*Streptomyces griseus*
Brevibacillus agri‡	*Enterococcus sanguinicola*†	*Propionibacterium granulosum*‡	*Veillonella atypica*‡
Brevibacillus brevis†‡	*Enterococcus thailandicus*	*Proteus mirabilis**‡	*Veillonella dispar*‡
Brevibacillus choshinensis	*Escherichia coli**‡	*Proteus vulgaris*†	*Veillonella parvula*‡
Brevibacillus formosus	*Eubacterium tenue*†‡	*Pseudomonas luteola*†	*Weissella cibaria*‡
Brevibacterium casei†‡	*Fusobacterium periodonticum*‡	*Pseudomonas oryzihabitans*†	

*species frequently associated with human disease[19].
†species rarely associated with human disease[19].
‡species already described in humans gut using culturomics[16–18].
§new bacterial species found in this study.

However, *M. tuberculosis, Treponema pallidum, B. anthracis, Streptococcus pneumoniae, Bordetella pertussis, Neisseria* spp. and *Francisella tularensis* were not detected via rtPCR in any fecal sample (Table 2).

Discussion

In this study, we used the culturomics approach because it has greater sensitivity than molecular methods and can be used to detect even minority bacterial populations including pathogens[16–18]. Many opportunistic human pathogens were observed using culturomics, including 8 pathogens frequently associated with human disease (Table 1)[19,31]. Among these, *C. botulinum* is also present in herbivore and carnivore hosts, including pigs, horses, cattle, giant pandas and tigers[32–36]. In contrast, *M. bolletii* is an emerging pathogen for which no animal source is known[31].

Our molecular examination of gorilla feces via real-time PCR confirmed these observations and demonstrated that wild gorillas are important reservoirs of emerging human pathogens, including fastidious bacteria such as *C. burnetii*, *Bartonella* spp., *T. whipplei* and *Borrelia* spp. (Table 2). This is the first observation of *T. whipplei* in a non-human primate host. Humans are the predominant reservoir and source of this bacterium[37]. Moreover, a high prevalence of pathogens, including *K. pneumoniae*, *Borrelia* spp., *S. aureus*, *P. aeruginosa* and *S. marcescens* was observed (Table 2).

The presence of these pathogens in samples from different sources collected at different periods as well as their high prevalence indicate that they are part of the commensal microbiota of the gorilla gut (Table 2)[12,15]. It is important to consider whether these opportunistic human pathogens are virulent under certain conditions in gorillas. The pathogenicity of certain bacteria in gorillas and chimpanzees has been observed; these bacteria include *B. anthracis*-like bacteria, *S. aureus*, *M. leprae* and *Pasteurella multocida*[11–13,38].

Furthermore, new culture media were developed based on the herbivore behavior of gorillas; these media were generated using 4 different plants. We observed that applying adapted culture media enhances the growth of bacteria that are difficult to cultivate. For example, rumen extracts mimicking the gut habitat were used to isolate a high proportion of human gut microbiota[16]. Here, approximately one-fifth of the recovered bacterial species were isolated specifically in the novel media supplemented with plant extracts, and more than one-third of all observed species, including 2 new species, grew in these media (Supplementary Table 2). It will be important to test other gorilla fecal samples and to develop further novel media using other plants eaten by gorillas, for instance, to mimic the ecological conditions that can promote bacterial growth[39].

Table 2 | Human pathogenic bacteria targeted via Real-Time PCR in 48 fecal samples (21 individuals) from wild gorillas

Real time PCR for	Target	Number of Positive fecal samples	Number of positive gorillas	Prevalence (%)
Klebsiella pneumoniae	Hemolysin	32	17	80.95%
Acinetobacter spp.	rpoB	43	16	76.19%
Borrelia spp.	16S rRNA	16	10	47.6%
Staphylococcus aureus	NucA	11	9	42.86%
Bartonella spp.	ITS	13	8	38.10%
Pseudomonas aeruginosa	oprL	10	8	38.10%
Serratia marcescens	16S rRNA	10	7	33.33%
Mycobacterium abscessus	gyrB	10	6	28.57%
Enterococcus faecalis	recN	6	6	28.57%
Coxiella burnetii	Hypotheticalprotein	5	5	23.80%
Escherichia coli	ompG	4	4	19.00%
Acinetobacter baumannii	OXA51	4	4	19.00%
Tropheryma whipplei	WiSPfamilyprotein	1	1	4.76%
Rickettsia africae	ITS	0	0	0
Enterococcus faecium	SodA	0	0	0
Mycobacterium tuberculosis	ITS	0	0	0
Streptococcus pneumoniae	PlyNR	0	0	0
Treponema pallidum	polA	0	0	0
Neisseria gonorrhoeae	Hypotheticalprotein	0	0	0
Neisseria meningitidis	Ctra	0	0	0
Bacillus anthracis	ANT	0	0	0
Bordetella pertussis	IS481	0	0	0
Francisella tularensis	yqaB	0	0	0
Mycoplasma pneumoniae	ITS	0	0	0

In conclusion, we observed that many human bacterial species, including pathogens, are also present in the gorilla gut. This type of non-invasive analysis of gorilla feces could facilitate the surveillance and discovery of potential human pathogens and unknown bacteria, especially in areas where human and gorilla habitats overlap and because of the increasing presence of humans in the African equatorial forests. Furthermore, similar studies on non-human primates would contribute to the detection of emerging diseases circulating in wild fauna.

Methods

Samples. Gorillas' fecal samples from two sites in south-central Cameroon (near the Minton village and near the Messok village) were collected as previously described[40] (about 1 g of dung was collected in a 50-ml tube containing RNAlater™ (Ambion, Austin, TX)) and preserved at ambient temperature for a maximum of 3 weeks in the field and subsequently stored at −80°C, except the sample used in culturomics, for which about 25 g of dung was collected and stored directly at −80°C without the RNAlater reagent. The time between defecation and collection was estimated at <24 h, according to the physical texture of the samples. No experimentation was conducted on these animals. The collection of fecal samples from the soil was approved by the Ministry of Scientific Research and Innovation of Cameroon. No other permit was required, as this research was non-invasive work, and the collection of the samples did not disrupt wild fauna.

Microsatellite analyses. The DNA extracted from 48 fecal samples was used for microsatellite analysis as previously described[40]. Briefly, seven loci including D18S536, D4S243, D10S676, D9S922, D2S1326, D2S1333 and D4S1627 were amplified (Supplementary Table 1). A region of amelogenin was also amplified for determining the gender[40]. The loci were amplified four times to exclude allelic dropout. One microliter of the amplification product was mixed with 10 µl of formamide and 0.25 µl of size marker (ROX GeneScan 400HD, Applied Biosystems, Foster city, CA). This mixture was analyzed via sequencing using the 3130xl Genetic Analyzer (Applied Biosystems). The sequencing products were visualized using the GeneMapper 3.7 software (Applied Biosystems). The number of wild gorillas was then identified by comparing the loci in the 48 samples analyzed (Supplementary Table 1).

Culturomics and bacterial identification. Two strategies were used in this study. First, 70 conditions were selected, which were effective for detecting more than 370 bacterial species, including minority pathogens[16] (Supplementary Table 2). Second, new media were developed including solid media supplemented with plants. Tobacco leaves and the fruits of mango, banana and papaya were crushed and lyophilized, and solutions containing 12 mg of plant extract per ml of sterile water were prepared and filtered using 0.2-µm filters. Additionally, a solution of 14 mg of agar per ml of sterile water was prepared. Using these solutions, the following media were prepared: filtered mango solution/agar (20 ml/80 ml); filtered papaya solution/agar (20 ml/80 ml); filtered banana solution/agar (10 ml/900 ml); filtered tobacco solution/agar (10 ml/90 ml); 12 g of lyophilized tobacco + 5% sheep blood (BioMerieux, Craponne, France)/agar (14 g/l); and 12 g of lyophilized banana + 5% sheep blood (BioMerieux, Craponne, France)/agar (14 g/l).

Additionally, culture vials (BD BACTEC™ Plus Aerobic/F Medium and BD BACTEC™ Lytic/10 Anaerobic/F Medium) supplemented with 5 ml of filtered banana solution were used. Finally, we also used MOD4 agar medium (specific for the culture of *Mycobacterium* spp.)[23] and 12 g of soil from the Timone hospital garden (Latitude: N 43° 17′ 20.151″; Longitude: E 5° 24′ 15.3822″)/agar (14 g/l).

For the fecal sample collected near Minton, the stool was divided into 1-g aliquots. Each gram was suspended in 9 ml of Dulbecco's Phosphate-Buffered Saline 1× (DPBS) and diluted from 1/10 to $1/10^{10}$; appropriate dilutions of the stool samples were plated onto different culture media at various conditions (Supplementary Table 2). The colonies isolated in different media were identified using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) and, when necessary, using 16S rRNA gene sequencing as previously reported[16].

New bacterial species. The new species *Gorillibacterium massiliense, Paenibacillus gorillae, Bacillus massiliogorillae, Microbacterium gorillae* and *Paenibacillus camerounensis* were deposited in the German collection of microorganisms (DSMZ) under the accession numbers DSM 27179, DSM 26181, DSM 26159, DSM 26203 and DSM 26182, respectively, and were submitted in the GenBank database under the accession numbers KC193239, JX650054, JX650055, JX650056 and JX650057, respectively. The description and genome sequencing of these new species were performed for three of them[20–22] and are ongoing for those remaining.

454 FLX Titanium pyrosequencing analyses. DNA was extracted from the feces as previously described (Macherey-Nagel, Hoerdet, France). 16S rRNA amplicon pyrosequencing and pyrosequencing analyses were performed as previously reported[16]. A sequence file was deposited to the NCBI Sequence Read Archive under the run accession number SRR1177866 and BioProject accession number PRJNA239545.

Real-time PCR assay for the detection of human pathogens. The primers and probes used in this study were previously described or designed in our laboratory for routine diagnosis (Supplementary Table 3). The DNA was extracted from 48 gorilla fecal samples using a kit (Macherey-Nagel, Hoerdet, France) as previously described[16]. In each PCR, 5 µl of DNA of each fecal sample was used in a final volume of 25 µl using the QuantiTect Probe PCR Kit (Qiagen, Courtaboeuf, France). The real-time PCR was performed using a CFX96™ Real-Time PCR Detection System (Bio-Rad, Life Science, Marnes-la-Coquette, France) with the following program: 95°C for 15 min followed by 44 cycles of 95°C for 0.5 min and 60°C for 1 min. The positive samples were sequenced using the same primers.

1. Duval, L. & Ariey, F. Ape *Plasmodium* parasites as a source of human outbreaks. *Clin. Microbiol. Infect.* **18**, 528–532 (2012).

2. Leroy, E. M. *et al.* Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science* **303**, 387–390 (2004).
3. Liu, W. *et al.* Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. *Nature* **467**, 420–425 (2010).
4. Peeters, M. & Delaporte, E. Simian retroviruses in African apes. *Clin. Microbiol. Infect.* **18**, 514–520 (2012).
5. Raoult, D. The apes as reservoir of human pathogens. *Clin. Microbiol. Infect.* **18**, 513 (2012).
6. Sharp, P. M., Rayner, J. C. & Hahn, B. H. Great apes and zoonoses. *Science* **340**, 284–286 (2013).
7. Calvignac-Spencer, S., Leendertz, S. A., Gillespie, T. R. & Leendertz, F. H. Wild great apes as sentinels and sources of infectious disease. *Clin. Microbiol. Infect.* **18**, 521–527 (2012).
8. Bermejo, M., Rodriguez-Teijeiro, J. D., Illera, G., Barroso, A., Vila, C. & Walsh, P. D. Ebola outbreak killed 5000 gorillas. *Science* **314**, 1564 (2006).
9. Kalema-Zikusoka, G., Rothman, J. M. & Fox, M. T. Intestinal parasites and bacteria of mountain gorillas (*Gorilla beringei beringei*) in Bwindi Impenetrable National Park, Uganda. *Primates* **46**, 59–63 (2005).
10. Keita, A. K. *et al.* Molecular evidence for the presence of *Rickettsia Felis* in the feces of wild-living African apes. *PLoS One* **8**, e54679 (2013).
11. Klee, S. R. *et al.* Characterization of *Bacillus anthracis*-like bacteria isolated from wild great apes from Côte d'Ivoire and Cameroon. *J. Bacteriol.* **188**, 5333–5344 (2006).
12. Nagel, M. *et al.* Human-associated *Staphylococcus aureus* strains within great ape populations in Central Africa (Gabon). *Clin. Microbiol. Infect.* **19**, 1072–1077 (2013).
13. Meyers, W. M., Gormus, B. J., Walsh, G. P., Baskin, G. B. & Hubbard, G. B. Naturally acquired and experimental leprosy in nonhuman primates. *Am. J. Trop. Med. Hyg.* **44**, 24–27 (1991).
14. Coscolla, M. *et al.* Novel *Mycobacterium tuberculosis* complex isolate from a wild chimpanzee. *Emerg. Infect. Dis.* **19**, 969–76 (2013).
15. Rwego, I. B., Isabirye-Basuta, G., Gillespie, T. R. & Goldberg, T. L. Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. *Conserv. Biol.* **22**, 1600–1607 (2008).
16. Lagier, J. C. *et al.* Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin. Microbiol. Infect.* **18**, 1185–1193 (2012).
17. Dubourg, G. *et al.* The gut microbiota of a patient with resistant tuberculosis is more comprehensively studied by culturomics than by metagenomics. *Eur. J. Clin. Microbiol. Infect. Dis.* **32**, 637–645 (2013).
18. Pfleiderer, A., Lagier, J. C., Armougom, F., Robert, C., Vialettes, B. & Raoult, D. Culturomics identified 11 new bacterial species from a single anorexia nervosa stool sample. *Eur. J. Clin. Microbiol. Infect. Dis.* **32**, 1–11 (2013).
19. Mandell, G. L., Bennett, J. E. & Dolin, R. Principles and practice of infectious diseases. *Elsevier* **7**th ed. (2010).
20. Keita, M. B. *et al.* Non-contiguous finished genome sequence and description of *Gorillibacterium massiliense* gen. nov, sp. nov., a new member of the family *Paenibacillaceae*. *Stand. Genomic Sci.* **9** (2014).
21. Keita, M. B., Diene, S. M., Robert, C., Raoult, D., Fournier, P. E. & Bittar, F. Non-contiguous finished genome sequence and description of *Bacillus massiliogorillae* sp. nov. *Stand. Genomic. Sci.* **9**, 93–105 (2013).
22. Keita, M. B. *et al.* Non-contiguous finished genome sequence and description of *Paenibacillus gorillae* sp. nov. *Stand. Genomic Sci.* **9** (2014).
23. Ghodbane, R., Raoult, D. & Drancourt, M. Dramatic reduction of culture time of *Mycobacterium tuberculosis*. *Sci. Rep.* **4**, 4236 (2014).
24. Adekambi, T., Berger, P., Raoult, D. & Drancourt, M. *rpoB* gene sequence-based characterization of emerging non-tuberculous mycobacteria with descriptions of *Mycobacterium bolletii* sp. nov., *Mycobacterium phocaicum* sp. nov. and *Mycobacterium aubagnense* sp. nov. *Int. J. Syst. Evol. Microbiol.* **56**, 133–143 (2006).
25. Frey, J. C., Rothman, J. M., Pell, A. N., Nizeyi, J. B., Cranfield, M. R. & Angert, E. R. Fecal bacterial diversity in a wild gorilla. *Appl. Environ. Microbiol.* **72**, 3788–3792 (2006).
26. Vlckova, K., Mrazek, J., Kopecny, J. & Petrzelkova, K. J. Evaluation of different storage methods to characterize the fecal bacterial communities of captive western lowland gorillas (*Gorilla gorilla gorilla*). *J. Microbiol. Methods* **91**, 45–51 (2012).
27. Yildirim, S. *et al.* Characterization of the fecal microbiome from non-human wild primates reveals species specific microbial communities. *PLoS One* **5**, e13963 (2010).
28. Szekely, B. A., Singh, J., Marsh, T. L., Hagedorn, C., Werre, S. R. & Kaur, T. Fecal bacterial diversity of human-habituated wild chimpanzees (*Pan troglodytes schweinfurthii*) at Mahale Mountains National Park, Western Tanzania. *Am. J. Primatol.* **72**, 566–574 (2010).
29. Bo, X. *et al.* Phylogenetic analysis of the fecal flora of the wild pygmy loris. *Am. J. Primatol.* **72**, 699–706 (2010).
30. Peris-Bondia, F., Latorre, A., Artacho, A., Moya, A. & D'Auria, G. The active human gut microbiota differs from the total microbiota. *PLoS One* **6**, e22448 (2011).
31. Sassi, M., Ben Kahla, I. & Drancourt, M. *Mycobacterium abscessus* multispacer sequence typing. *BMC Microbiol.* **13** (2013).
32. Böhnel, H., Wagner, C. & Gessler, F. Tonsils-Place of botulinum toxin production: results of routine laboratory diagnosis in farm animals. *Vet. Microbiol.* **130**, 403–409 (2008).
33. Johnson, A. L., McAdams, S. C. & Whitlock, R. H. Type A botulism in horses in the United States: A Review of the past Ten Years (1998–2008). *J. Vet. Diagn. Invest.* **22**, 165–173 (2010).
34. Myllykoski, J., Nevas, M., Lindström, M. & Korkeala, H. The detection and prevalence of *Clostridium botulinum* in pig intestinal samples. *Int. J. Food Microbiol.* **110**, 172–177 (2006).
35. Williams, J. H., Bester, L., Venter, L., Pretorius, D. & Greyling, F. Barbiturate ingestion in three adult captive tigers (*Panthera tigris*) and concomitant fatal botulism of one. *J S Afr Vet Assoc.* **82**, 244–249 (2011).
36. Zhu, L., Wu, Q., Dai, J., Zhang, S. &Wei, F. Evidence of cellulose metabolism by the giant panda gut microbiome. *Proc. Natl. Acad. Sci. U. S. A* **108**, 17714–17719 (2011).
37. Keita, A. K. *et al.* Looking for *Tropheryma whipplei* source and reservoir in rural Senegal. *Am. J. Trop. Med. Hyg.* **88**, 339–343 (2013).
38. Kondgen, S. *et al. Pasteurella multocida* involved in respiratory disease of wild chimpanzees. *PLoS One* **6**, e24236 (2011).
39. Ley, R. E. *et al.* Evolution of mammals and their gut microbes. *Science* **320** (5883), 1647–1651 (2008).
40. Neel, C. *et al.* Molecular epidemiology of simian immunodeficiency virus infection in wild-living gorillas. *J. Virol.* **84**, 1464–1476 (2010).

Acknowledgments

Fadi BITTAR was supported by a Chair of Excellence IRD provided by the Institut de Recherche pour le Développement/IHU Méditerranée Infection. We thank Amandine Esteban for microsatellite analyses. This work was supported in part by grants from the National Institute of Health (RO1 AI 50529) and the Agence Nationale de Recherches sur le SIDA (ANRS 12255).

Author contributions

D.R. and F.B. designed the experiments; M.K. conducted the experiments; F.B., M.K., J.C.L., M.P., E.D. and D.R. analyzed the results; F.B., M.K. and D.R. wrote the manuscript. All authors reviewed the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Bittar, F. *et al. Gorilla gorilla gorilla* gut: a potential reservoir of pathogenic bacteria as revealed using culturomics and molecular tools. *Sci. Rep.* **4**, 7174; DOI:10.1038/srep07174 (2014).