MALDI-TOF Identification of the Human Gut Microbiome in People with and without Diarrhea in Senegal

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Abstract

Background: In Africa, there are several problems with the specific identification of bacteria. Recently, MALDI-TOF mass spectrometry has become a powerful tool for the routine microbial identification in many clinical laboratories.

Methodology/Principal Findings: This study was conducted using feces from 347 individuals (162 with diarrhea and 185 without diarrhea) sampled in health centers in Dakar, Senegal. Feces were transported from Dakar to Marseille, France, where they were cultured using different culture conditions. The isolated colonies were identified using MALDI-TOF. If a colony was unidentified, 16S rRNA sequencing was performed. Overall, 2,753 isolates were tested, allowing for the identification of 189 bacteria from 5 phyla, including 2 previously unknown species, 11 species not previously reported in the human gut, 10 species not previously reported in humans, and 3 fungi. 2,718 bacterial isolates (98.8%) out of 2,750 yielded an accurate identification using mass spectrometry, as did the 3 Candida albicans isolates. Thirty-two bacterial isolates not identified by MALDI-TOF (1.2%) were identified by sequencing, allowing for the identification of 2 new species. The number of bacterial species per fecal sample was significantly higher among patients without diarrhea (8.6 ± 3) than in those with diarrhea (7.3 ± 3; P = 0.0003). A modification of the gut microbiota was observed between the two groups. In individuals with diarrhea, major commensal bacterial species such as Escherichia coli were significantly decreased (85% versus 64%), as were several Enterococcus spp. (E. faecium and E. casseliflavus) and anaerobes, such as Bacteroides spp. (B. uniformis and B. vulgatus) and Clostridium spp. (C. bifermentans, C. orbiscindens, C. perfringens, and C. symbiosum). Conversely, several Bacillus spp. (B. licheniformis, B. mojavensis, and B. pumilus) were significantly more frequent among patients with diarrhea.

Conclusions/Significance: MALDI-TOF is a potentially powerful tool for routine bacterial identification in Africa, allowing for a quick identification of bacterial species.

Introduction

There are several problems in the specific identification of bacterial infections in Africa. Currently, bacterial identification is based on phenotypic tests, including Gram staining, bacterial culture, culture growth characteristics, and biochemical profiles. Even if culture processes are available in major hospitals in Africa, there are limitations to the performance of biochemical identification methods. Such traditional methods require the possession of many API strips including API-20E, API-20NE, API Staph kits, and API Anaerobe kits and many unique reagents that should be stocked under specific conditions and have expiration dates. Biochemical methods are time consuming. They are often required knowledge about the type of microorganism being tested, and fail to accurately identify several bacteria species [1,2].

Five years ago, a revolution occurred in bacteriology with the advent of the routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) [1,3–5]. Currently, this technique allows accurate identification of bacteria without a priori knowledge of the type of microorganism. This technique is in widespread use in many clinical laboratories in Europe [1,3,4,6,7]. This method allows for the detection of bacteria in less than 1 hour and is cost effective. Thus, this technique has become a powerful tool for routine identification and could replace Gram staining and biochemical identification, but to this point, many studies using this technique have been mainly performed in Europe [2].

The bacterial repertoire is different depending on the environment from which the microorganisms are obtained [8,9]. For example, differences at the species level have been observed among the microbes in the human between Asian versus American people and European versus African people [10,11]. Another recently developed high-throughput method involves the combination of culturomics using a large panel of media incubated at several atmospheric conditions and MALDI-TOF mass spectrometry for the quick and accurate identification of a large number of colonies [12–14].
In this study, we evaluated the effectiveness of MALDI-TOF mass spectrometry on the identification of bacterial species isolated from feces from Senegalese patients with and without diarrhea by combining several culture conditions and rapid mass spectrometry identification.

Materials and Methods

Ethics Statement

All aspects of this study were approved by the National Ethical Committee (CNERS) of Senegal (SEN25/07). Written consent was obtained for all participants. For children, their parents or guardians provided also a written informed consent.

Patient Recruitment and Sample Management

This study was based on 347 individuals, adults and children, sampled from March 2009 to January 2010: 162 individuals with diarrhea and 185 without diarrhea (Table 1). Five health centers in Dakar, Senegal and its suburbs (Dominique-Pikine, Sicap Miao, Roi Baudoin, Institut d’Hygiène Sociale, and Saint Martin) were included. Stool samples were collected from children and adults who attended these health centers. Control patients were hospitalized patients or outpatients without intestinal pathogens or recent treatment with antibiotics.

Stool specimens were collected in special sterile stool containers or with swabs for stool samples collected from infants. All stool samples were labeled and transported in cool boxes for examination within 24 hours of collection to Institut Pasteur de Dakar (Senegal). At the laboratory, macroscopic and microscopic analyses were performed on fresh stool samples to look for enteric pathogens including eggs, cysts, and trophozoites of intestinal parasites as well as enteric viruses.

Culturomics Methods

To enumerate the number of colony forming units (CFU) in the stool samples, 1 g of pasty stool was diluted in 9 ml of phosphate buffered saline (PBS), and 100 μl of watery stool was diluted in 900 μl of PBS. The diluted samples were introduced with a syringe for preincubation into aerobic and anaerobic blood culture bottles (BD Bactec Plus Lytic/10 Anaerobic, Aerobic, 39 Heidelberg, Germany) for 24 hours before being inoculated on agar plates as it was still not accurately identified by MALDI-TOF after two attempts, the isolate was analyzed by 16S rRNA sequencing.

Identification Using Mass Spectrometry

The isolated colonies were deposited on a MALDI-TOF target microflex (Bruker Daltonik, Wissembourg, France) and overlaid with matrix solution, a saturated solution of 2-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid, after air-drying at room temperature for 5 minutes. Each colony was picked from an Eppendorf tube containing the Trypcticase-Casein-Soy (AES) culture medium stored at 37°C. Broth culture-specific thiglycollate (BD Diagnostics) was used for anaerobes. Two spots were examined for each colony. Each deposit was covered with 2 μl of the matrix solution. The Biotyper software was used to compare the protein profile of the bacteria obtained from a database (Bruker and the base of the Timone hospital) of protein profiles regularly updated based on the results of clinical diagnosis. This software takes into account a maximum of 100 mass peaks between 3,000 and 15,000 Da. A score >1.9 indicates a high-level identification of genus and species. A score >1.7 indicates the identification of genus but not species, and a score lower than 1.7 indicates no identification of bacteria. If the species was not still accurately identified by MALDI-TOF after two attempts, the isolate was analyzed by 16S rRNA sequencing.

16S rRNA Amplification and Sequencing Identification

Bacterial DNA was extracted using the MagNA Pure LC kit DNA isolation kit III (Roche, France) with the MagNA Pure LC instrument, according to the manufacturer’s instructions. The 16S rRNA gene was amplified by PCR using the universal primer pair fd1 and rp2 and an annealing temperature of 52°C, as described elsewhere [16]. PCR products were purified using the PCR kit Nucleofast 96 (Macherey-Nagel, Hoerdt, France). Sequencing reactions were performed with the sequencing kit Big Dye Terminator version 1.1 (Perkin-Elmer, Coignieres, France). Sequencing reactions were analyzed on an ABI PRISM 3130X Genetic Analyzer (Applied Biosystems, California, USA). The obtained sequences were compared with the GenBank database using BLAST software. A threshold value of similarity ≥98.7% was used for identification at the species level. Below this value, sequences were repeated to confirm the first obtained results. A new species was

Table 1. Population description.

| Age (years) | Patients | Controls |
|-------------|----------|----------|
| 0–5         | 71       | 9        |
| 5–20        | 35       | 46       |
| ≥20         | 56       | 130      |
| Total       | 162      | 185      |

|      | Number | %     | Number | %     |
|------|--------|-------|--------|-------|
| 0–5  | 71     | 43.8  | 9      | 4.9   |
| 5–20 | 35     | 21.6  | 46     | 24.9  |
| ≥20  | 56     | 34.6  | 130    | 70.2  |
| Total| 162    | 100.0 | 185    | 100.0 |

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suspected when the similarity in the GenBank database with described bacteria was 98.7% [17,18].

Statistical Analyses
Statistical analyses were performed using EpiInfo6 software (http://www.cdc.gov/epiinfo/Epi6/EI6dnjp.htm). The results were concluded to be statistically significant when $P<0.05$. The corrected chi-squared test or Fisher’s exact test was used where indicated.

Results

Culture
Overall, 2,753 isolates were tested, which allowed us to identify 189 bacterial species from 5 phyla, including an unknown species and 3 fungi (Table 4 and Figure 1) [19]. Two stool specimens from patients with diarrhea did not allow for the recovery of any bacteria. *Candida albicans* was detected from 3 patients with diarrhea (3/162 versus 0/185, $P=0.1$). A total of 1,175 bacterial isolates were detected among patients with diarrhea and 1,575 were detected among patients without diarrhea. The number of different bacterial species per stool sample was significantly higher among patients without diarrhea (mean of 8.6±3.4, range 1 to 18) than among those with diarrhea (mean of 7.3±3.4, range 0 to 22; $P=0.0003$). Finally, 59 out of the 153 bacterial species (38.6%) identified among patients with diarrhea were specific for this group whereas 36 out of the 129 bacterial species (27.9%) identified among patients without diarrhea were specific for this group, although this difference is not significant ($P=0.059$).

MALDI-TOF Mass Spectrometry Identification
Of the 2,750 bacterial isolates analyzed, 2,718 (98.8%) yielded an accurate identification using MALDI-TOF mass spectrometry (Table 4).

16S rRNA Amplification and Sequencing Identification
Thirty-two isolates out of the 2,750 (1.2%) were not identified by MALDI-TOF mass spectrometry. Among these isolates, 11 were identified using 16S rRNA sequencing: *Bacteroides nordii*, *Bacillus clausii*, *Bacillus thuringiensis*, *Clostridium cadaveris*, *Clostridium neonatale*, *Paenibacillus polymyxa*, *Staphylococcus sciuri*, *Shigella boydii*, *Shigella sonnei*, and two new species were identified: a new clostridial species that was called *Clostridium dakarense* sp. nov. (GenBank accession number KC517358) and a new *Bacillus* species, *Bacillus casamencensis* sp. nov. (GenBank accession number AF519462.1). The 16S rRNA sequence of this *Bacillus* species has been already detected in rice soils in Senegal but no description of the bacterium has been yet reported. The full genome of *C. dakarense* has been recently sequenced and reported [20].

Table 2. Culture media and conditionings used in this study.

| Media            | Culture conditions | Suppliers                  |
|------------------|--------------------|----------------------------|
| Direct inoculation |                    |                            |
| 5% sheep blood agar | Aerobe, 37°C, 48 hours | Biomerieux, Marcy l’Etoile, France |
| 5% sheep blood agar | Anaerobe, 37°C, 48 hours | Biomerieux                  |
| MacConkey        | Aerobe, 37°C, 48 hours | Biomerieux                  |
| BCYE             | Aerobe with 2.5% CO$_2$, 37°C, 5 days | Biomerieux                  |
| BCP              | Aerobe, 37°C, 48 hours | Biomerieux                  |
| LAMVAB           | Anaerobe, 37°C     | Home-made*                 |

Inoculation in a blood culture bottle for 24 h, followed by inoculation in

| Culture conditions | Suppliers |
|-------------------|-----------|
| Columbia Aerobe, 37°C, 3 days | Biomerieux |
| MacConkey Aerobe, 37°C, 1 day | Biomerieux |
| Columbia Anaerobe, 37°C, 3 days | Biomerieux |

BCYE: Buffered Charcoal Yeast Extract; BCP: Bromocresol Purple; LAMVAB: Lactobacillus Anaerobic MRS with Vancomycin and Bromocresol green. *from Hartemink et al. [15].

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Table 3. Primers used for 16S rRNA PCR and sequencing.

| Primers | Sequences (5’–3’) | Annealing temperature |
|---------|--------------------|-----------------------|
| FD1     | AGAGTTTGATCCTGGCTCAG | 52°C                  |
| RP2     | ACCGCTACTTAGTACGACTT | 52°C                  |
| 536F    | CAGCAGGGGGCTTAAC     | 50°C                  |
| 536R    | GTTATACCCGGCTCTTG    | 50°C                  |
| 800F    | ATTAGATACCCTGGTAG    | 50°C                  |
| 800R    | CTACCAAGGATATCTAAT   | 50°C                  |
| 1050F   | TGTCGTCAGCTGGTG      | 50°C                  |
| 1050R   | CAGACGTGACGACAGA     | 50°C                  |

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The other isolates identified by 16S rRNA sequence included 1 of 2 Parabacteroides goldsteinii isolates detected in the study, 1 of 2 Aneurinibacillus migulanus isolates, 2 (11%) of 18 Bacillus amyloliquefaciens isolates, 1 of 2 Bacillus endophyticus isolates, 1 (7.7%) of 13 Bacillus licheniformis isolates, 2 (7.7%) of 26 Bacillus pumilus isolates, 5 (8%) of 37 Bacillus subtilis, 1 of 4 Clostridium clostridioforme isolates, 1 of 13 (7.7%) Clostridium litusebrense isolates, 1 of 13 (7.7%) Kurthia gibsonii isolates, 1 of 4 Lactococcus lactis isolates, 1 of 25 (4%) Lysinibacillus fusiformis isolates, 1 of 2 Lysinibacillus sphaericus isolates, 1 of 2 Ruminococcus gnavus isolates, 1 of 12 (8.3%) Weissella cibaria isolates, and 2 of 11 (18.2%) Akinetobacter baumannii isolates. When the spectra of the aforementioned isolates were added to the Bruker database, further identifications of these organisms by MALDI-TOF were accurate.

**Common bacteria.** Seven bacterial species (3.7%) were identified more than 100 times in fecal samples (261 Escherichia coli isolates, 256 Enterococcus faecium isolates, 159 Clostridium bifermentans isolates, 153 Enterococcus faecalis isolates, 152 Clostridium perfringens isolates, 137 Bacillus cereus isolates, and 106 Enterococcus hirae isolates). Surprisingly, several bacteria were more common in patients without diarrhea including E. coli than those without (P < 10^-3), E. faecium (P = 10^-5), C. bifermentans (P = 0.002), and C. perfringens (P = 10^-3); see Table 4 and Figure 2.

Thirty-nine bacterial species (20.6%) from 18 different genera were identified from between 10 and 100 fecal samples (Table 4 and Figure 2). Several were more common in patients with diarrhea than those without, such as Bacillus licheniformis (P = 0.02), Bacillus pumilus (P = 0.002), and Staphylococcus aureus (P = 0.01). In contrast, people without diarrhea had more commonly Lysinibacillus fusiformis (P = 0.001), Clostridium oryctolagus (P = 0.01), Clostridium symbiosum (P = 0.03), Enterococcus casseliflavus (P = 0.03), Kurthia gibsonii (P = 0.02), and Collinsella aerofaciens (P = 0.01), Eggerthella lenta (P = 0.004), Bacteroides uniformis (P = 0.001), and Bacteroides vulgatus (P = 0.03).

**Rare bacterial species.** Overall, 81 out of 189 bacterial species (43%) were identified from between 2 and 10 fecal samples (Table 4). Among them, Bifidobacterium breve, Propionibacterium acnes, Bacillus mojavensis, Fusobactera magna, and Streptococcus anginosus were each detected in only 4 patients with diarrhea (P = 0.047). Staphylococcus haemolyticus was detected in only 5 patients with diarrhea (P = 0.02). Staphylococcus epidermidis was significantly more frequent among people with diarrhea (7/162) than among those without (1/185, P = 0.02). In contrast, Enterobacteriaceae limosum was identified only in 5 people without diarrhea (P = 0.04).

**Bacterial species isolated only once.** Overall, 51 bacterial species were identified only once (Table 4). Among them, 5 different bacterial species from the phylum Actinobacteria, 5 from the genera Bacillus, 4 from the genera Clostridium, and 2 from the genera Shigella were detected among patients with diarrhea. In contrast, several species of the genera Bacteroides (4) and Enterococcus (2) were detected only among patients without diarrhea.

**Bacterial identification depending of the age range.** The isolates obtained from people with and without diarrhea depending of the age range (less than 5 years, from 5 to 20 years, and more than 20 years) were compared. Only significant differences are presented (Table S1). For children from 0 to 5 year-old, 2 species of the genera Clostridium were significantly more frequent among those without diarrhea, including 1 species C. glycocolicum, for which the data were not significant when the entire population was analyzed. For adult of more than 20 year-old, 6 species (E. coli, E. faecium, B. uniformis, B. vulgatus, C. oryctolagus, and E. lenta), as previously observed in the entire population, were significantly more observed in people without diarrhea. In contrast, those with diarrhea had more commonly S. aureus, E. magna, B. pumilus, as previously observed, as well as another Bacillus species, B. subtilis. For people from 5 to 20 year-old, E. faecium, C. perfringens, and C. symbiosum were significantly more detected in people without diarrhea, as observed in the entire population. Finally, the comparison of the isolates from people with diarrhea between them depending of the age range did not yield statistically significant results.
### Table 4. Comparison between the prevalence of 189 bacterial species identified among 2,750 isolates from fecal samples of 162 individuals with diarrhea and 185 without diarrhea.

| Phyla          | Bacteria                          | 162 with diarrhea | 185 without diarrhea | Total = 347 |
|----------------|-----------------------------------|-------------------|-----------------------|-------------|
|                | N’ of isolate | %                | N’ of isolate | %                | N’ of isolate | %                | P value   |
|>100            |                    |                  |                      |                  |                  |                  |
| Proteobacteria | Escherichia coli | 104              | 64.2                | 157              | 84.9            | 261              | 75.2       | ≤10⁻³      |
| Firmicutes     | Enterococcus faecium | 102              | 63                  | 154              | 83.2            | 256              | 73.8       | ≤10⁻³      |
| Firmicutes     | Clostridium bifermentans | 60               | 37                  | 99               | 53.5            | 159              | 45.8       | 0.002      |
| Firmicutes     | Enterococcus faecalis | 76               | 46.9                | 77               | 41.6            | 153              | 44         | ns          |
| Firmicutes     | Clostridium perfringens | 53               | 32.7                | 99               | 53.5            | 152              | 43.8       | ≤10⁻³      |
| Firmicutes     | Bacillus cereus | 57               | 35.2                | 80               | 43.2            | 137              | 39.5       | ns          |
| Firmicutes     | Enterococcus hirae | 48               | 29.7                | 58               | 31.3            | 106              | 30.5       | ns          |
|>10-100         |                    |                  |                      |                  |                  |                  |           |
| Firmicutes     | Enterococcus gallinarum | 34               | 21                  | 52               | 28.1            | 86               | 24.8       | ns          |
| Proteobacteria | Klebsiella pneumoniae | 33               | 20.4                | 51               | 27.6            | 84               | 24.2       | ns          |
| Firmicutes     | Clostridium sordelli | 29               | 17.9                | 48               | 25.9            | 77               | 22.2       | ns          |
| Firmicutes     | Lactococcus garvieae | 23               | 14.2                | 34               | 18.4            | 57               | 16.4       | ns          |
| Bacteroidetes  | Bacteroides fragilis | 20               | 12.5                | 35               | 18.9            | 55               | 15.8       | ns          |
| Firmicutes     | Enterococcus avium | 20               | 12.3                | 32               | 17.3            | 52               | 14.5       | ns          |
| Firmicutes     | Clostridium orbiscindens | 12               | 7.4                 | 30               | 16.2            | 42               | 12.1       | 0.01       |
| Proteobacteria | Enterobacter cloacae | 23               | 14.2                | 18               | 9.7             | 41               | 11.8       | ns          |
| Bacteroidetes  | Bacteroides uniformis | 8                | 5                   | 30               | 16.2            | 38               | 10.9       | 0.001      |
| Firmicutes     | Bacillus subtilis | 22               | 13.6                | 15               | 8.1             | 37               | 10.7       | ns          |
| Firmicutes     | Clostridium symbiosum | 10               | 6.2                 | 25               | 13.5            | 35               | 10         | 0.03       |
| Firmicutes     | Enterococcus casseliflavus | 10              | 6.2                 | 25               | 13.5            | 35               | 10         | 0.03       |
| Bacteroidetes  | Bacteroides thetaiotaomicron | 9               | 5.5                 | 21               | 11.3            | 30               | 8.6        | ns          |
| Firmicutes     | Streptococcus equinus | 13               | 8                   | 16               | 8.6             | 29               | 8.4        | ns          |
| Actinobacteria | Collinsella aerofaciens | 6                | 3.7                 | 20               | 10.8            | 26               | 7.5        | 0.01       |
| Firmicutes     | Bacillus pumilus | 19               | 11.7                | 7                | 3.8             | 26               | 7.5        | 0.002      |
| Firmicutes     | Streptococcus lutetiansis | 16              | 9.9                 | 10               | 5.4             | 26               | 7.5        | ns          |
| Firmicutes     | Lysinibacillus fusiformis | 4                | 2.5                 | 21               | 11.3            | 25               | 7.2        | 0.001      |
| Bacteroidetes  | Bacteroides ovatus | 10               | 6                   | 14               | 7.6             | 24               | 6.9        | ns          |
| Firmicutes     | Streptococcus galolyticus | 16              | 9.9                 | 8                | 4.3             | 24               | 6.9        | ns          |
| Proteobacteria | Proteus mirabilis | 9                | 5.6                 | 15               | 8.1             | 24               | 6.9        | ns          |
| Actinobacteria | Eggerthella lenta | 4                | 2.5                 | 19               | 10.3            | 23               | 6.6        | 0.004      |
| Proteobacteria | Comamonas kerstieri | 8                | 4.9                 | 12               | 6.5             | 20               | 5.8        | ns          |
| Firmicutes     | Clostridium butyricum | 6                | 3.7                 | 13               | 7               | 19               | 5.5        | ns          |
| Firmicutes     | Clostridium glycolyces | 5                | 3                   | 14               | 7.6             | 19               | 5.5        | ns          |
| Bacteroidetes  | Bacteroides vulgatus | 2                | 1.2                 | 16               | 8.7             | 18               | 5.2        | ≤10⁻³      |
| Firmicutes     | Bacillus amylofiquefaciens | 10              | 6.2                 | 8                | 4.3             | 18               | 5.2        | ns          |
| Firmicutes     | Clostridium tertium | 11               | 6.8                 | 7                | 3.8             | 18               | 5.2        | ns          |
| Firmicutes     | Clostridium coheleanum | 4                | 2.5                 | 12               | 6.5             | 16               | 4.6        | ns          |
| Bacteroidetes  | Parabacteroides distasonis | 7                | 4.3                 | 8                | 4.3             | 15               | 4.3        | ns          |
| Proteobacteria | Morganella morganii | 6                | 3.7                 | 9                | 4.9             | 15               | 4.3        | ns          |
| Firmicutes     | Bacillus licheniformis | 10               | 6.2                 | 3                | 1.6             | 13               | 3.7        | 0.02       |
| Firmicutes     | Clostridium lituseburensis | 4              | 2.5                 | 9                | 4.9             | 13               | 3.7        | ns          |
| Firmicutes     | Kurthia gibsonii | 2                | 1.2                 | 11               | 5.9             | 13               | 3.7        | 0.02       |
| Firmicutes     | Clostridium ramorum | 5                | 3                   | 7                | 3.8             | 12               | 3.5        | ns          |
| Firmicutes     | Staphylococcus aureus | 10               | 6.2                 | 2                | 1               | 12               | 3.5        | 0.01       |
| Firmicutes     | Weissella cibaria | 4                | 2.5                 | 8                | 4.3             | 12               | 3.5        | ns          |
Table 4. Cont.

| Phyla     | Bacteria                                | 162 with diarrhea | 185 without diarrhea | Total = 347 |
|-----------|-----------------------------------------|-------------------|----------------------|-------------|
|           | N° of isolate | %                  | N° of isolate | %                  | N° of isolate | %                  | P value |
| Proteobacteria | Acinetobacter baumannii² | 4 | 2.5 | 3 | 3.8 | 7 | 3.8 | 11 | 4 | ns |
| Firmicutes | Streptococcus parasanguinis | 8 | 4.9 | 3 | 1.6 | 11 | 3.2 | ns |
| 1–10 isolates | Bacillus circulans | 5 | 3 | 5 | 2.7 | 10 | 2.9 | ns |
| Firmicutes | Bacillus weihenstephanensis | 5 | 3 | 4 | 2.2 | 9 | 2.6 | ns |
| Firmicutes | Enterococcus thailandicus² | 3 | 1.8 | 6 | 3.2 | 9 | 2.6 | ns |
| Firmicutes | Streptococcus pneumoniae | 5 | 3 | 4 | 2.2 | 9 | 2.6 | ns |
| Firmicutes | Enterococcus canintestini | 4 | 2.5 | 4 | 2.2 | 8 | 2.3 | ns |
| Firmicutes | Enterococcus durans | 6 | 3.7 | 2 | 1 | 8 | 2.3 | ns |
| Firmicutes | Staphylococcus epidermidis | 7 | 4.3 | 1 | 0.5 | 8 | 2.3 | 0.02 |
| Actinobacteria | Micrococcus luteus | 5 | 3 | 2 | 1 | 7 | 2 | ns |
| Firmicutes | Bacillus siralis | 3 | 1.8 | 4 | 2.2 | 7 | 2 | ns |
| Firmicutes | Enterococcus dispar | 4 | 2.5 | 3 | 1.6 | 7 | 2 | ns |
| Firmicutes | Enterococcus raffinosus² | 3 | 1.8 | 4 | 2.2 | 7 | 2 | ns |
| Proteobacteria | Enterobacter hormaechei | 5 | 3 | 2 | 1 | 7 | 2 | ns |
| Firmicutes | Aneurinibacillus aneurinilyticus | 2 | 1.2 | 4 | 2.2 | 6 | 1.7 | ns |
| Firmicutes | Clostridium sporogenes | 4 | 2.5 | 2 | 1 | 6 | 1.7 | ns |
| Firmicutes | Streptococcus agalactiae | 4 | 2.5 | 2 | 1 | 6 | 1.7 | ns |
| Firmicutes | Streptococcus dysgalactiae² | 5 | 3 | 1 | 0.5 | 6 | 1.7 | ns |
| Proteobacteria | Citrobacter freundii | 1 | 0.6 | 5 | 2.7 | 6 | 1.7 | ns |
| Firmicutes | Eubacterium limosum | 0 | 0 | 5 | 2.7 | 5 | 1.4 | 0.04 |
| Firmicutes | Pseudobacillus pueri | 2 | 1.2 | 3 | 1.6 | 5 | 1.4 | ns |
| Firmicutes | Staphylococcus haemolyticus | 5 | 3 | 0 | 0 | 5 | 1.4 | 0.02 |
| Firmicutes | Streptococcus salivarius | 4 | 2.5 | 1 | 0.5 | 5 | 1.4 | ns |
| Proteobacteria | Acinetobacter calcoaceticus | 2 | 1.2 | 3 | 1.6 | 5 | 1.4 | ns |
| Proteobacteria | Escherichia fergusonii | 3 | 1.8 | 2 | 1 | 5 | 1.4 | ns |
| Proteobacteria | Klebsiella oxytoca | 2 | 1.2 | 3 | 1.6 | 5 | 1.4 | ns |
| Actinobacteria | Bifidobacterium breve | 4 | 2.5 | 0 | 0 | 4 | 1.1 | 0.047 |
| Actinobacteria | Propionibacterium acnes | 4 | 2.5 | 0 | 0 | 4 | 1.1 | 0.047 |
| Firmicutes | Bacillus majovenis | 4 | 2.5 | 0 | 0 | 4 | 1.1 | 0.047 |
| Firmicutes | Clostridium clostridiforme | 3 | 1.8 | 1 | 0.5 | 4 | 1.1 | ns |
| Firmicutes | Clostridium hadleyi | 3 | 1.8 | 1 | 0.5 | 4 | 1.1 | ns |
| Firmicutes | Clostridium paraputrificum | 3 | 1.8 | 1 | 0.5 | 4 | 1.1 | ns |
| Firmicutes | Enterococcus asini | 0 | 0 | 4 | 2.2 | 4 | 1.1 | ns |
| Firmicutes | Finegoldia magna | 4 | 2.5 | 0 | 0 | 4 | 1.1 | 0.047 |
| Firmicutes | Lactococcus lactis² | 1 | 0.6 | 3 | 1.6 | 4 | 1.1 | ns |
| Firmicutes | Streptococcus anginosus | 4 | 2.5 | 0 | 0 | 4 | 1.1 | 0.047 |
| Proteobacteria | Enterobacter asburiae | 0 | 0 | 4 | 2.2 | 4 | 1.1 | ns |
| Proteobacteria | Haemophilus parainfluenzae | 3 | 1.8 | 1 | 0.5 | 4 | 1.1 | ns |
| Proteobacteria | Pseudomonas aeruginosa | 2 | 1.2 | 2 | 1 | 4 | 1.1 | ns |
| Proteobacteria | Salmonella enterica | 3 | 1.8 | 1 | 0.5 | 4 | 1.1 | ns |
| Firmicutes | Lactobacillus gasseri | 3 | 1.8 | 0 | 0 | 3 | 0.9 | ns |
| Firmicutes | Lactobacillus plantarum | 1 | 0.6 | 2 | 1 | 3 | 0.9 | ns |
| Firmicutes | Pseudobacillus jamilae² | 3 | 1.8 | 0 | 0 | 3 | 0.9 | ns |
| Firmicutes | Pseudobacillus larvae² | 3 | 1.8 | 0 | 0 | 3 | 0.9 | ns |
| Firmicutes | Staphylococcus capitis | 2 | 1.2 | 1 | 0.5 | 3 | 0.9 | ns |
| Firmicutes | Staphylococcus hominis | 2 | 1.2 | 1 | 0.5 | 3 | 0.9 | ns |
Table 4. Cont.

| Phyla       | Bacteria                        | 162 with diarrhea | 185 without diarrhea | Total = 347 |
|-------------|---------------------------------|--------------------|-----------------------|-------------|
|             | N° of isolate | %                  | N° of isolate | %            | N° of isolate | %      | P value |
| Firmicutes  | Staphylococcus lugdunensis      | 3 1.8              | 0 0            | 3 0.9        | ns           |
| Firmicutes  | Staphylococcus pasteuri         | 2 1.2              | 1 0.5          | 3 0.9        | ns           |
| Firmicutes  | Streptococcus alactolyticus     | 0 0                | 3 1.6          | 3 0.9        | ns           |
| Proteobacteria | Enterobacter kobei        | 0 0                | 3 1.6          | 3 0.9        | ns           |
| Proteobacteria | Acinetobacter schindleri    | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Actinobacteria | Bifidobacterium catenulatum   | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Actinobacteria | Bifidobacterium longum       | 0 0                | 2 1            | 2 0.6        | ns           |
| Bacteroidetes | Parabacteroides goldsteinii | 0 0                | 2 1            | 2 0.6        | ns           |
| Bacteroidetes | Parabacteroides johnsonii    | 0 0                | 2 1            | 2 0.6        | ns           |
| Firmicutes  | Aneurinibacillus migulanus     | 2 1.2              | 0 0            | 2 0.6        | ns           |
| Firmicutes  | Bacillus badius                | 0 0                | 2 1            | 2 0.6        | ns           |
| Firmicutes  | Bacillus endophyticus         | 2 1.2              | 0 0            | 2 0.6        | ns           |
| Firmicutes  | Bacillus megaterium           | 2 1.2              | 0 0            | 2 0.6        | ns           |
| Firmicutes  | Bacillus pseudomycoidei       | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Firmicutes  | Clostridium aldenense         | 0 0                | 2 1            | 2 0.6        | ns           |
| Firmicutes  | Clostridium difficile         | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Firmicutes  | Clostridium indolcis          | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Firmicutes  | Clostridium innocuum          | 2 1.2              | 0 0            | 2 0.6        | ns           |
| Firmicutes  | Clostridium subterminale      | 2 1.2              | 0 0            | 2 0.6        | ns           |
| Firmicutes  | Clostridium tetani            | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Firmicutes  | Enterococcus canis            | 0 0                | 2 1            | 2 0.6        | ns           |
| Firmicutes  | Enterococcus cecorum          | 2 1.2              | 0 0            | 2 0.6        | ns           |
| Firmicutes  | Enterococcus pseudaoxidans    | 0 0                | 2 1            | 2 0.6        | ns           |
| Firmicutes  | Enterococcus tenue            | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Firmicutes  | Lysinibacillus sphaericus     | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Firmicutes  | Paeonibacillus alvei          | 0 0                | 2 1            | 2 0.6        | ns           |
| Firmicutes  | Pediococcus acidiputens       | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Firmicutes  | Ruminococcus gnavus           | 2 1.2              | 0 0            | 2 0.6        | ns           |
| Firmicutes  | Streptococcus infantarius     | 0 0                | 2 1            | 2 0.6        | ns           |
| Firmicutes  | Streptococcus mitis           | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Firmicutes  | Streptococcus oralis          | 2 1.2              | 0 0            | 2 0.6        | ns           |
| Firmicutes  | Streptococcus sanguinis       | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Proteobacteria | Acinetobacter radioresistens | 0 0                | 2 1            | 2 0.6        | ns           |
| Proteobacteria | Citrobacter koseri           | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Proteobacteria | Citrobacter sedlakii         | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Proteobacteria | Klebsiella variicola          | 1 0.6              | 1 0.5          | 2 0.6        | ns           |
| Proteobacteria | Proteus vulgaris              | 2 1.2              | 0 0            | 2 0.6        | ns           |
| 1 isolate   | Arthrobacter polyfornacogenes | 1 0.6              | 0 0            | 1 0.3        | ns           |
| Actinobacteria | Arthrobacter oxydans         | 1 0.6              | 0 0            | 1 0.3        | ns           |
| Actinobacteria | Bifidobacterium pseudacatenulatum | 1 0.6 | 0 0 | 1 0.3 | ns |
| Actinobacteria | Corynebacterium acerihomans | 1 0.6              | 0 0            | 1 0.3        | ns           |
| Actinobacteria | Corynebacterium striatum      | 1 0.6              | 0 0            | 1 0.3        | ns           |
| Bacteroidetes | Alistipes indistinctus        | 0 0                | 1 0.5          | 1 0.3        | ns           |
| Bacteroidetes | Alistipes onderdonkii         | 1 0.6              | 0 0            | 1 0.3        | ns           |
| Bacteroidetes | Bacteroides caccae           | 0 0                | 1 0.5          | 1 0.3        | ns           |
| Bacteroidetes | Bacteroides cellulinoslyticus | 0 0                | 1 0.5          | 1 0.3        | ns           |
| Phyla          | Bacteria                                | N° of isolate | %  | N° of isolate | %  | Total = 347 | %  | P value |
|---------------|-----------------------------------------|---------------|----|---------------|----|-------------|----|---------|
| Bacteroidetes | Bacteroides finegoldii                  | 162           | 0  | 185           | 0  |               |    |         |
| Bacteroidetes | Bacteroides intestinaleis               | 0             | 0  | 0             | 0  |               |    |         |
| Bacteroidetes | Bacteroides nordii1                     | 0             | 0  | 0             | 0  |               |    |         |
| Bacteroidetes | Peptoniphilus harei                    | 0             | 0  | 0             | 0  |               |    |         |
| Firmicutes    | Abiotrophia defectiva                  | 0             | 0  | 0             | 0  |               |    |         |
| Firmicutes    | Anaerotruncus colihominis               | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Bacillus casamancensis1,4               | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Bacillus clausii1                       | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Bacillus flexus                        | 0             | 0  | 0             | 0  |               |    |         |
| Firmicutes    | Bacillus koreensis2                     | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Bacillus marisflavi                    | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Bacillus mycoides                      | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Bacillus nordii1                       | 0             | 0  | 0             | 0  |               |    |         |
| Firmicutes    | Bacillus simplex                       | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Bacillus thuringiensis1                | 0             | 0  | 1             | 0.5|               |    |         |
| Firmicutes    | Bacillus coccoides                     | 0             | 0  | 0             | 0  |               |    |         |
| Firmicutes    | Bacillus agri                          | 0             | 0  | 1             | 0.5|               |    |         |
| Firmicutes    | Bacillus formosus2                     | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Clostridium baratii                    | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Clostridium cadaveris2                 | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Clostridium dakarense1,4               | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Clostridium irregular3                 | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Clostridium neonata1                   | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Clostridium senegalense                | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Enterococcus hermanniensis2            | 0             | 0  | 1             | 0.5|               |    |         |
| Firmicutes    | Enterococcus mundtii                   | 0             | 0  | 1             | 0.5|               |    |         |
| Firmicutes    | Gemella haemolysans                    | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Granulicatella adiacens                | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Granulicatella elegans                 | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Lactobacillus salivarius               | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Paenibacillus barcinonensis            | 0             | 0  | 1             | 0.5|               |    |         |
| Firmicutes    | Paenibacillus motobuenensis2           | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Paenibacillus polympyx2,3              | 0             | 0  | 1             | 0.5|               |    |         |
| Firmicutes    | Pediococcus pentosaceus                | 0             | 0  | 1             | 0.5|               |    |         |
| Firmicutes    | Staphylococcus cohnii                  | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Staphylococcus saprophyticus           | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Staphylococcus sciuri1                 | 0             | 0  | 1             | 0.5|               |    |         |
| Firmicutes    | Staphylococcus warneri                 | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Streptococcus constellatus             | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Turicibacter sanguinis                 | 1             | 0.6| 0             | 0  |               |    |         |
| Firmicutes    | Veillonella parvula                    | 1             | 0.6| 0             | 0  |               |    |         |
| Fusobacteria  | Fusobacterium varium                  | 0             | 0  | 1             | 0.5|               |    |         |
| Proteobacteria| Acinetobacter lwoffii2                 | 0             | 0  | 1             | 0.5|               |    |         |
| Proteobacteria| Citrobacter braakii                   | 1             | 0.6| 0             | 0  |               |    |         |
| Proteobacteria| Enterobacter aerogenes                 | 1             | 0.6| 0             | 0  |               |    |         |
| Proteobacteria| Enterobacter ludwigii1                 | 0             | 0  | 1             | 0.5|               |    |         |
| Proteobacteria| Kluyvera georgiana1                    | 0             | 0  | 1             | 0.5|               |    |         |
**Table 4. Cont.**

| Phyla         | Bacteria                   | 162 with diarrhea | 185 without diarrhea | Total = 347 |
|---------------|----------------------------|-------------------|-----------------------|-------------|
|               | N° of isolate | % | N° of isolate | % | N° of isolate | % | P value |
| Proteobacteria| Niesseria flavescens    | 1 | 0.6 | 0 | 0 | 1 | 0.3 ns |
| Proteobacteria| Proteus penneri        | 1 | 0.6 | 0 | 0 | 1 | 0.3 ns |
| Proteobacteria| Pseudomonas luteola    | 1 | 0.6 | 0 | 0 | 1 | 0.3 ns |
| Proteobacteria| Pseudomonas putida     | 0 | 0   | 0 | 1 | 0.5 | 0.3 ns |
| Proteobacteria| Shigella boydi          | 1 | 0.6 | 0 | 0 | 1 | 0.3 ns |
| Proteobacteria| Shigella sonnei        | 1 | 0.6 | 0 | 0 | 1 | 0.3 ns |

*P* value is specified only when a significant difference was observed.

N° of isolate: Number of isolate; %: Percentage; ns: non significant value.

1 Bacterial species that were never isolated in humans;
2 Bacterial species isolated in humans but not in the human gut;
3 New bacterial species.

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**Viral and Parasites Identification**

Analyses in Dakar have allowed the detection of several viruses and parasites in feces. Ten rotaviruses (6.2%), 4 adenoviruses (2.7%), and 7 co-infections with both rotaviruses and adenoviruses (4.3%) were detected among diarrheic patients. Sixteen Enterobius vermicularis (9.9%), 6 Trichomonas intestinalis (3.7%), 5 Cryptosporidium spp. (3%), 5 cysts of Entamoeba histolytica (3%), 4 Schistosoma mansoni (2.7%), and 1 Microsporidium spp. (0.6%) were detected among 37 diarrheic people. Thirty-six Ascaris lumbricoides (among 24 diarrheic people and 2 without diarrhea), 8 Giardia duodenalis (among 6 diarrheic people and 2 without diarrhea), and 4 Trichuris trichiura (among 1 diarrheic people and 3 without) were detected. Finally, 2 co-infections (Cryptosporidium spp. with Ascaris lumbricoides and Microsporidium spp. with Ascaris lumbricoides) were detected in patients with diarrhea and 1 co-infection (Trichuris trichiura with Ascaris lumbricoides) among a people without diarrhea.

**Discussion**

MALDI-TOF mass spectrometry coupled with culturomics has allowed for the identification of a large collection of bacterial species from specimens from Senegal and a preliminary comparison between the bacterial microbiota of people with and without diarrhea. This technique has allowed for the accurate identification of a large panel of anaerobes that are usually poorly identified by current phenotypic methods, which lack specificity and result in ambiguous or even erroneous identification [21,22]. For several bacterial species, their identification by MALDI-TOF failed because either the corresponding species missed in the database or either the number of spectra of the species was insufficient. Indeed, the continuous increases of the entries in database with the addition of our new spectra solved these problems and improved bacterial identification. In addition, the use of MALDI-TOF mass spectrometry detects the presence of previously rare bacteria that were difficult to identify using phenotypic methods [6,23–27].

Overall, the percentage of isolates from Senegal that were correctly identified at the genus and species level by mass spectrometry (98.2%) is nearly the same than the percentage (95.4%) observed in the first large scale experiment that used mass spectrometry in Marseille, France [1]. Both studies were performed using the same database. This study has allowed us to test a large collection of isolated strains from Senegalese people. Only 3 bacterial species, *Clostridium senegalense*, *Bacillus casamanceensis*, and *Clostridium daurosense*, have been currently identified in Senegal. This confirms the high potential for culturomics approaches to result in the detection of new bacterial species associated with humans [28–39]. The increases in the database by the addition of more bacteria have allowed for improved bacterial identification by MALDI-TOF mass spectrometry. Thus, the current database seems accurate for the identification of bacteria in Senegal. This work allowed for the identification of 166 bacterial species already found in the human gut, 11 species previously detected in humans but not in the gut, 10 species detected in humans for the first time, and 2 unknown species.

The composition of the gut microbiota is complex [40]. A recent culturomics experiment using many culture conditions was performed on fecal samples from 2 healthy Senegalese individuals, 1 obese person, 1 person with resistant tuberculosis, and a patient with anorexia nervosa. This allowed the identification of 99, 219, 192, and 133 different bacterial species per fecal sample, respectively [12–14]. Although the storage and transport conditions of the fecal samples were not optimal and many fewer culture conditions were used, this study demonstrates a modification of gut microbiota with several significant differences between the bacterial species identified among people with diarrhea and those without diarrhea. In people with diarrhea, major commensal bacterial species such as *E. coli* were significantly decreased, as were several *Enterococcus* spp. (*E. faecium* and *E. casseliflavus*); anaerobes, such as *Bacteroides* spp. (*B. uniformis* and *B. vulgatus*); and *Clostridium* spp. (*C. bifermentans*, *C. orbiscindens*, *C. peffringens*, *C. symbiosum*, and *C. glycolicum*). Conversely, several *Bacillus* spp. (*B. licheniformis*, *B. mucogenic*), *P. putida* and *B. subtilis*) were significantly more frequent among patients with diarrhea. In addition, the diversity of *Bacillus* species identified in patients with diarrhea is higher (19) than among those without diarrhea (11), but this difference was not significant (*P* = 0.055). Overall, a decrease of anaerobes in the gut flora, particularly *Bacteroidetes*, has already been reported during gastroenteritis using both culture and molecular methods [41,42]. Our data shows the occurrence of an imbalance of natural bacterial flora among patients with diarrhea.

Gut Microbiome in People in Senegal
For a long time, the high cost of a MALDI-TOF apparatus and the lack of specific reagent have limited the development of this technology. The expense of using MALDI-TOF mass spectrometry for identification now lies in the acquisition of a machine, which costs between €100,000 and €200,000 [21]. Recently, the cost per sample was calculated to be 1.35 euros for the Microflex system from Bruker [21]. The time required for bacterial identification has been improved to 1 minute 46 seconds using the Microflex system. In addition, MALDI-TOF mass spectrometry also has the potential for identification at the serotype level and antibiotic resistance profiling within minutes [43–51]. Thus, the rapid and accurate identification of routinely encountered bacterial species can be performed to improve the care of patients with infectious diseases. This technique will be a promising alternative for bacterial identification in Africa. Indeed, the main cost is based on the investment of purchasing the apparatus. The used reagents do not expire, do not require specific storage conditions, and are not expensive [1,6]. Finally, the protocol that involves directly deposited bacterial colonies onto the MALDI-TOF mass spectrometry plate regardless of the agar-based medium and without any subculture or colony preparation is very simple and can be widely used.

Overall, MALDI-TOF mass spectrometry is a potentially powerful tool for routine bacterial identification in Africa, as it allows for the rapid identification of bacterial species, including those that are rare and difficult to identify using phenotypic methods. The next step will be to install MALDI-TOF mass spectrometers in African hospitals.

**Supporting Information**

Table S1 Summary of the significant differences observed between the prevalence of bacterial species from fecal samples of 347 individuals with and without diarrhea depending of the age range.

(DOCX)

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**Author Contributions**

Conceived and designed the experiments: AGS FF DR. Performed the experiments: BSB JCL PH GD. Analyzed the data: CM HR FF DR. Wrote the paper: FF JCL DR.

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