Genomic characterization of fecal microbial communities in severely sick humans and broiler chickens using next generation sequencing

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Abstract

Background: Microbiota plays an important role in food safety and its alteration poses a serious threat to humans. Comparative microbiome profiling using next-generation sequencing (NGS) enabled the understanding of microbial diversity and similarity between different species. In this study, we used NGS to profile the fecal microbiota of sick human and broiler chickens. A total of 26 fecal samples were collected from severely sick human subjects (n= 13) and broiler chickens (n=13) with similar symptoms.

Results: The total number of microbial species detected in broiler chickens fecal microbiota was higher than that of humans. Phylum Proteobacteria was the most abundant in both human and broiler chickens fecal microbiota while Tenericutes was found to be least abundant in both species. Phylum Actinobacteria was found only in the human fecal microbiota. In both humans and broiler chickens, E.coli was found to be phylogenetically related suggesting a microbial association between both species.

Conclusion: NGS based taxonomic profiling revealed the association of microbial dysbiosis with extreme sickness in both humans and broiler chickens. The dominance of phylum Proteobacteria in both the species ascertains their altered gut microbiota. Both human and broiler chickens microbial communities were found to be genetically related indicating horizontal transfer of microbes between the two species.

Background

With the advent of next-generation sequencing technology (NGS), microbial communities within the gut microbiome of human and farm animals have been studied extensively [1]. Until now, microbial communities within the gut of human and various animals were studied using culture-dependent methodologies which hampered the growth of anaerobic bacteria [2-3]. Previously, the majority of intestinal bacteria were considered 'unculturable' using standard laboratory culture conditions [4]. The normal human gut microbial communities are dominated by three anaerobic bacteria phyla including *Bacteriodes*, *Firmicutes*, and *Actinobacteria* [5]. Similarly, *Firmicutes*, *Proteobacteria,* and *Bacteroidetes* have been identified as the three dominated bacterial phyla within the poultry gut microbial communities [6]. The other important taxonomic groups present within the gut-microbiome of human are *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia*, methanogenic archaea, and various other diverse eukaryotic communities such as protists and fungi [7]. A number of microbes transiently colonize gut conditions. Until now, the important roles of the human microbiome in health and diseases were not clear due to the limitations of standard culture-based techniques [8]. However, high throughput sequencing fast-tracked gut microbiome research [9]. Currently, 16S rDNA amplicon and shotgun metagenomic sequencing are the two common approaches used to address different aspects of gut microbiota in various pathological conditions including inflammatory bowel disease, diabetes, and obesity [10]. In comparison with 16S rDNA amplicon sequencing, shotgun metagenomic offers greater sensitivity, specificity, microbial abundance estimation, and greater resolution of microbes up to the species level [11]. Evidence support the fact that various pathogenic microbes can be transmitted from animals to humans or from humans to animals either through direct contact or while living with livestock

in close contact and it has been exemplified by a recent report indicating the transmission of antibiotic-resistant bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Staphylococcus aureus*, and various members of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* [12-14]. Currently, foodborne infections pose a serious health threat to human life and such infections are transmitting from food-producing animals to humans via the food chain [15]. Among the various foodborne pathogens, *Salmonella, Campylobacter, Listeria, Escherichia, Shigella, Yersinia,* and *Vibrio* are the major microbial genera causing significant morbidity and mortality across the globe [16, 17]. The human gut is the reservoir of trillions of microbes comprising of not only normal flora but a variety of pathogenic bacteria and the great majority of these pathogens are zoonotic [18]. Broiler chickens are considered as an economic alternative of red-meat in Pakistan and are consumed heavily across the country [19]. Antibiotics as growth promoters are banned in various developed countries [20] however, in Pakistan, various antibiotics are still frequently used as feed additives [21]. Recent chicken microbiome studies documented that multiple factors including feed alterations, the composition of litter, antibiotics, the addition of probiotics to the feed, various diseases and stress are linked with alteration of their gut-microbiome [22, 23]. According to the One Health approach, the transmission of various pathogenic microbes among different hosts is due to the existence of an ecological relationship between humans, animals, and environmental health [24]. Investigation of microbial transfer between humans, various animals, and the environment is needed while applying One Health concept to the microbiome-based studies [25, 26]. Understanding the association of various microbiomes between different species and their environment can reveal novel approaches related to diagnosis, treatment, and intervention of several deadly diseases [27]. Keeping in view the One Health approach, we attempted to profile the fecal microbiota of human (HS) and broiler chickens (BC) using NGS shotgun metagenomic sequencing. Besides comparative microbial profiling, one of our objectives was to ascertain the phylogenetic relatedness of bacterial species between humans and poultry.

Results

Selection of samples with similar symptoms for sequencing

On the basis of similar symptoms, we selected 26 samples (13 each) out of 1000 screened samples for shotgun metagenomic sequencing of HC and BC fecal microbiota.

Microbial taxonomy

Microbial profiling of HS and BC fecal samples was carried out using *MEGAN6* software [67]. The microbial profiles of dominated phyla, families, and genera of HS and BC fecal samples are shown in Figures 3 and 4, respectively.

Microbial profiling at the phylum level

In total, 5 phyla were identified in HS fecal samples namely *Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria,* and *Tenericutes*. Conversely, 4 phyla were identified in BC fecal samples namely

Proteobacteria, Firmicutes, Bacteroidetes, and *Tenericutes*. The fecal microbial profile of HS samples showed the presence of *Actinobacteria* whereas this specific phylum was not found in BC fecal samples. Among all these phyla, *Proteobacteria* was the most dominated phylum in both HS and BC fecal samples accounted for 45.6% and 38.9%, respectively. In HS samples, the second most abundant phylum was *Bacteroidetes* (36.6%), followed by *Firmicutes* (12.5%), *Actinobacteria* (5%), and *Tenericutes* (0.3%). In BC fecal samples, the second most abundant phylum was *Firmicutes* (36.4%), followed by *Bacteroidetes* (15.8%) and *Tenericutes* (8.9%).

Microbial profiling at the family level

In both HS and BC fecal samples, 10 microbial families were found to be the most abundant. Families dominated in HS fecal microbiota were *Enterobacteriaceae, Bacteroidaceae, Ruminococcaceae, Clostridiaceae, Bifidobacteriaceae, Enterococcaceae, Selenomonadaceae, Sphingobacteriaceae, Streptococcaceae and Aeromonadaceae.* In contrast to the HS fecal microbiota, families dominated in BC fecal samples were *Enterobacteriaceae, Bacteroidaceae, Clostridiaceae, Lactobacillaceae, Pseudomonadaceae, Acholeplasmataceae, Staphylococcaceae, Lachnospiraceae, Ruminococcaceae,* and *Caulobacteraceae.* Families specifically found only in HS fecal microbiota were *Bifidobacteriaceae, Enterococcaceae, Selenomonadaceae, Sphingobacteriaceae, Streptococcaceae,* and *Aeromonadaceae* whereas *Lactobacillaceae, Pseudomonadaceae, Acholeplasmataceae, Staphylococcaceae, Lachnospiraceae,* and *Caulobacteraceae* dominated only in BC fecal microbiota. Among all these families, *Enterobacteriaceae* was the most abundant in both HS and BC fecal samples, which accounted for 40% and 24.1% respectively. Similarly, family *Bacteroidaceae* was the second most abundant family in both HS and BC fecal samples, which accounted for 32% and 16.4% respectively. The third most abundant family in HS fecal microbiota was *Ruminococcaceae* (8.8%) followed by *Clostridiaceae* (5.1%), *Bifidobacteriaceae* (4.4%), *Enterococcaceae* (3.3%), *Selenomonadaceae* (1.8%), *Sphingobacteriaceae* (1.3%), *Streptococcaceae* (0.9%) and *Aeromonadaceae* (0.7%). Conversely, the third most abundant family in BC fecal samples was *Clostridiaceae* (11.7%) followed by *Lactobacillaceae* (11.4%), *Pseudomonadaceae* (10.9%), *Acholeplasmataceae* (8%), *Staphylococcaceae* (7.3%), *Lachnospiraceae* (5.2%), *Ruminococcaceae* (4.7%) and *Caulobacteraceae* (0.3%). Overall, 6 families were found unique in both HS and BC fecal samples.

Microbial profiling at the genus level

Microbial profiling revealed 10 abundant genera in both HS and BC fecal samples. Those included *Bacteroides, Escherichia, Enterobacter, Salmonella, Faecalibacterium, Ruminococcus, Alistipes, Bifidobacterium, Enterococcus, Coprococcus, Pseudomonas, Lactobacillus, Faecalibacterium, Phytoplasma* and *Staphylococcus*. Among these, genus *Bifidobacterium, Enterococcus* and *Coprococcus* dominated specifically only in HS samples while *Pseudomonas, Lactobacillus, Faecalibacterium, Phytoplasma,* and *Staphylococcus* dominated only in BC fecal samples. The most abundant genera in HS samples were *Bacteroides* (22.9%) followed by *Escherichia* (20.1%), *Enterobacter* (9.7%), *Salmonella* (9.1%), *Faecalibacterium* (7.1%), *Ruminococcus* (6.1%), *Alistipes* (4.8%), *Bifidobacterium* (3.1%),

Enterococcus (2.9%) and *Coprococcus* (2.7%). In contrast, the most abundant genera found in BC fecal microbiota were *Escherichia* (15.9%) followed by *Alistipes* (13.5%), *Bacteroides* (10.9%), *Pseudomonas* (10.9%), *Lactobacillus* (10%), *Faecalibacterium* (10.3%), *Phytoplasma* (8%), *Ruminococcus* (7.4%), *Staphylococcus* (6.9%) and *Salmonella* (6.4%).

Microbial profiling at the species level

The various abundant and common species found in HS and BC fecal samples are shown in Figures 5, 6 and 7 respectively. In total, 37 species were found in HS fecal microbiota while 58 species were found in BC fecal samples. Among the various species, *E.coli, Salmonella enterica, Klebsiella oxytoca, Klebsiella aerogenes, Klebsiella pneumoniae, Raoultella ornithinolytica, Bacteroides fragilis, Bacteroides thetaiotaomicron, Butyrate-producing bacterium SS3/4, Ruminococcus bromii, Ruminococcus champanellensis, Ruminococcus torques, Faecalibacterium prausnitzii, Alistipes shahii, Arsenophonus nasoniae, Shigella flexneri,* and *Enterobacter cloacae* were the unique species found HS fecal samples. In contrast, species found only BC fecal samples were *Shigella dysentriae, Shigella sp.SF-2015, Yersinia enterocolitica, Escherichia fergusonii, Blautia obeum A2-162, Butyrivibrio fibrisolvens, Ralstonia solanacearum, Marinobacter adhaerens, Achromobacter xylosoxidans, Pseudomonas fluorescens, Pseudomonas stutzeri, Pseudomonas putida, Pseudomonas aeruginosa, Staphylococcus xylosus, Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Staphylococcus warneri, Staphylococcus pettenkoferi, Solibacillus silvestris, Halobacillu shalophilus, Caulobacter vibrioides, Bacillus subtilis, Lactobacillus salivarius, Lactobacillus johnsonii, Lactobacillus helveticus.*

Phylogenetic analysis

Genetic analysis of *E.coli* found in both HS and BC fecal samples was carried out against the reference strain *E.coli K-12 substr. MG1655* (*NC 000913.3*) on the basis of sequence similarity. *E.coli* dominated in BC fecal microbial profile was found to be 99.48% similar to the reference *E.coli* strain. Conversely, *E.coli* dominated in HS fecal samples was found to be 98.2% similar to the reference strain. Upon comparing the sequence similarity of *E.coli* dominated in the HS and BC fecal samples with respect to each other, the similarity was found to be 98% (Figure 8).

Discussion

In this study, we used NGS shotgun metagenomics to profile HS and BC fecal microbiota of severely sick HS and BC with similar symptoms. Phylogenetic analysis was performed to investigate the bacterial association between humans and poultry. Metagenomic profiling of HS fecal microbiota revealed a predominance of phylum *Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria* while *Tenericutes* was the least dominant group. Similarly, BC fecal microbiota was dominated by *Proteobacteria, Firmicutes, Bacteroidetes* while *Tenericutes* constitute the least abundant group. We observed that *Proteobacteria*was dominantly present in both HS and BC fecal microbiota. The higher abundance of *Proteobacteria* in this study reflects the state of the altered gut microbiome of both humans and poultry [28, 29]. Our findings are in line with a previous study that reported a significantly higher abundance of

phylum *Proteobacteria* in the hospitalized Israeli population [30]. Similarly, another study found that the distribution of various bacterial phyla in pigs was altered after 14 days of antibiotics intake with a considerable increase in *Proteobacteria* in comparison with the group raised without antibiotics using the approach of 16S rDNA amplicon sequencing [31]. Furthermore, a recent study found a significantly higher percentage of *Proteobacteria* in fecal samples of both layers and BC speculating that antibiotic intake might be the contributing factor for the alteration of gut microbiota [32]. The predominance of *Proteobacteria* has been documented as a diagnostic marker of various diseases and dysbiosis [33]. An altered gut microbiome is termed as dysbiosis which may lead to various pathological conditions such as reducing the barrier function of the intestine, reduced nutrient digestive potential, increased risk of bacterial translocation and various inflammatory conditions [34]. In addition, the predominance of *Proteobacteria* has been reported to be associated with the emergence of opportunistic pathogens [35, 36].

During the taxonomic profiling of the samples, we observed that phylum *Actinobacteria* was present only in HS microbiota. The complete loss of *Actinobacteria* in BC fecal microbiota can be attributed to the high usage of antibiotics as growth promoters in BC feed. Our suspicion is supported by a recent report indicating a decrease in *Actinobacteria* in chicken fed with antibiotics as growth promoters [37, 38]. In our study, we observed that phylum *Tenericutes* was the least abundant in both HS and BC fecal microbiota. This could be explained by a recent report indicating that antibiotic treatment drastically affected the abundance of *Tenericutes* [39].

Looking at bacterial class level diversity, we found 10 different classes in HS fecal whereas only 6 classes were detected in BC fecal samples (Fig. 3 and 4). Various factors are reported to affect the diversity of gut microbiota including age, intestinal transit time, diet and use of antibiotics as growth promoters in chicken feed [40-42]. Mortality at an early age can possibly be a reason for lower microbial diversity in BC fecal microbiota. A recent report demonstrated the enrichment of intestinal microbial diversity with increasing age in chickens [6].

We found common useful and pathogenic genera between HS and BC fecal samples. Useful common genera in HS and BC fecal samples were *Bacteroides, Faecalibacterium, Alistipes, and Ruminococcus* while *Escherichia* and *Salmonella* were the common pathogenic genera. Our result of a greater abundance of *Bacteroides* and *Escherichia* in HS microbiota is in agreement with a previous report indicating a higher abundance of *Escherichia* and *Bacteroidetes in elderly subjects indicating gut* dysbiosis [43]. The results of this study based on the genera distribution of BC fecal microbiota are not in agreement with the previous studies [44-47]. The variation of our results from the previous studies could possibly be explained by the fact that their studies were based on 16S rDNA amplicon sequencing using healthy chickens raised under various conditions. In our study, the presence of a higher abundance of pathogenic genera in both HS and BC samples is in line with a recent report indicating that a higher abundance of the particular pathogen within the intestinal communities causes greater liability to be affected by those particular pathogens. Furthermore, a higher abundance of commensal *E. coli* was attributed to an increased level of *Salmonella enterica* infections [48].

A number of common useful and pathogenic bacterial species were identified at the species level in HS and BC fecal microbiota. Useful common species in HS and BC were *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Butyrate-producing bacterium SS3/4*, *Ruminococcusbromii*, *Ruminococcus champanellensis*, *Ruminococcus torques*, *Faecalibacterium prausnitzii*, and *Alistipes shahii* while common pathogenic bacteria were *E.coli*, *Salmonella enterica*, *Klebsiella oxytoca*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Raoultella ornithinolytica*, *Arsenophonus nasoniae*, *Shigella flexneri,* and *Enterobacter cloacae.*

The various pathogenic bacterial species identified in BC fecal microbiota have been reported to be zoonotic pathogens transmitted to humans through contaminated food [49-51]. Humans acquire the majority of foodborne infections upon direct or indirect exposure to contaminated food from a variety of host species including poultry, dogs, cats and livestock [52]. The co-dominance of various foodborne pathogens such as *E.coli* and *Salmonella enteric* in both HS and BC fecal microbiota indicates a possible route through which these pathogenic bacteria are transferred between the two species. Among the different pathogenic bacteria, *E.coli* has been considered as an important source of serious infections in both humans and poultry [53]. *E.coli* is the causative agent of infections designated as extraintestinal pathogenic *E. coli* (ExPEC) [54]. Among the various forms of ExPEC, avian-pathogenic *E.coli (*APEC) causes severe poultry infections collectively called as avian *Colibacillosis* [55]. Similarly, the intestinal tract of humans is considered as the main reservoir of ExPEC, however, multiple recent reports have indicated that BC can act as an external source of ExPEC transmitted to humans through the food chain [56]. *Our results based on phylogenetic analysis revealed that E.coli from BC and HS fecal samples was 99.48% and 98.2% similar to the reference strain, respectively. Furthermore, we found that E.coli from HS and BC fecal microbiota had 98% identity to each other. In the present study, similarity found through phylogenetic analysis between E.coli from HS and BC fecal microbiota might reflect the transmission of pathogenic E.coli from poultry to humans through the food chain. Our suspicion of E.coli transmission from poultry to humans is supported by few studies indicating that* avian pathogenic APEC and human ExPEC strains are phylogenetically related [57-58].

Conclusion

The alteration of gut microbiota with various factors is now a well-established fact. In this study, we compared the gut microbiome of severely sick humans and poultry using NGS. Our findings demonstrate the identification of *Proteobacteria* to be the most abundant phylum in both HS and BC fecal samples indicating an altered gut microbiome. Phylum *Actinobacteria* was found only in HS fecal samples providing a point of difference between the two species. Moreover, *Bacteroides* and *Escherichia* were the most abundant genera in HS and BC fecal samples respectively. Our findings indicate a higher number of bacterial species in BC than that of HS fecal samples. The presence of various pathogenic bacterial species in HS and BC fecal samples reflects their altered gut microbial communities. Furthermore, this study found that human and poultry *E.coli* are phylogenetically related indicating horizontal transfer of microbes between the two species.

Methods

Fecal samples were used for gut microbiome profiling of human and broiler chickens.

Collection of fecal samples

Initially, we screened 1000 samples for both human and poultry using the records of Microbiology Laboratory of Hayatabad Medical complex, and Veterinary Research Institute, Peshawar Pakistan. Fecal samples (n=26) were collected from the severely sick human subjects (n= 13, aged 45 years) and broiler chickens (n=13, aged 4-5 weeks) with similar symptoms under aseptic measures. Fecal samples were transported to the laboratory on ice and were mixed thoroughly using a spatula. *Aliquots* of 0.2g *were made and stored* at −80 °C.

DNA extraction

DNA from HS and BC fecal samples were extracted using PureLink^{TM} Microbiome DNA Purification Kit (Invitrogen, ThermoFisher Scientific; Cat. no. A29790) following manufacturer's instruction with modifications made to optimize the protocol according to the simple laboratory benchtop instruments (unpublished data). Genomic DNA concentration was determined using Qubit fluorometer following manufacturer's instructions (Qubit™ fluorometer, Invitrogen, CA 92008, USA) [59].

NGS library preparation

DNA was used to prepare NGS library using Illumina® Nextera XT DNA Library Preparation Kit (FC-131-1096, Illumina Inc., CA, USA) and Nextera XT Index Kit v2 Set A (FC-131-2001, Illumina Inc., CA, USA) by following manufacturer's instructions [60]. The generated DNA libraries were pooled and loaded onto the flow cell. Paired-end sequencing (2x150 bp) was performed using Illumina MiSeq (Illumina Inc., CA, USA) [61]. The reagent cartridge used for sequencing was *300 cycle V2 MiSeq reagent kit* (MS-103-1002, Illumina Inc., CA, USA).

Sequencing data analysis

Paired-end sequencing generated FASTQ files that were analyzed using various publicly available bioinformatics softwares. NGS sequenced data were analyzed through multiple steps as follows:

1. All FASTQ data were de-multiplexed using the CASAVA 8.2 package [62].
2. By using our custom data analysis pipeline, all the samples were analyzed for microbial characterization (Fig. 1).

3. Raw data comprising of low quality reads, adapters and technical biases were removed using *Trimmomatic* 0.36 [63].

4. All the microbial reads were decontaminated from human DNA using a computational tool Knead Data 0.6.1 [64].

5. *De-novo* assembly of microbes was carried out by using SPAdes assembler v.5.0 [65].

6. The microbial profiles were further aligned against NCBI's non-redundant protein reference database (NCBI-*NR*) using DIAMOND aligner v.0.9.25 [66].

7. *DIAMOND blast results were interpreted using MEtaGenome ANalyzer* (*MEGAN* 6) software for the taxonomic classification of microbes [67].

Genetic analysis

For genetic analysis, FASTQ files of various bacterial strains detected within the fecal samples of HS and BC were aligned against the reference genome. *Samtools mpileup v.1.9 was used to* generate FASTA files [68]. Using *ClustalW*, all the FASTA files were aligned against the reference genome and a phylogenic tree was constructed using the BioNJ distance algorithm [69, 70]. The complete bioinformatics workflow of genetic analysis is shown in Fig. 2.

Visualization of microbial profiles

Microbial community abundance was visualized using Pavain to generate cladograms for various taxonomic groups while hclust2 was used to generate heatmaps based on Bray-Curtis dissimilarity for sample clustering [71-72].

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Rehman Medical Institute-Research Ethics Committee (Ref: RMI/RMI-REC/Approval/33) Peshawar, Pakistan. Informed consent was obtained from the all patients who participated in this study.

Consent for publication

Not applicable

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

OKA performed experiments, analyzed data, wrote the paper. JA co-supervised and designed the study, provided advice in data analysis, critically discussed results and edited paper. JHC supervised the study, provided advice in study design and co-edited the paper.

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Figures
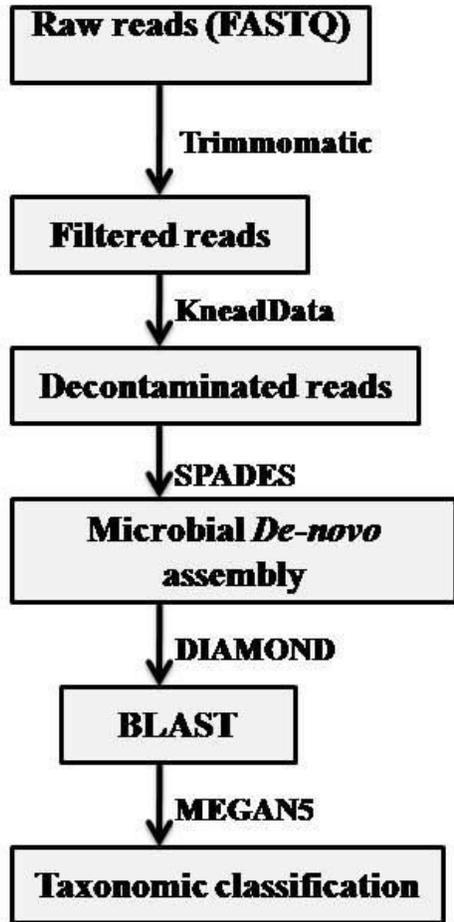
Figure 1

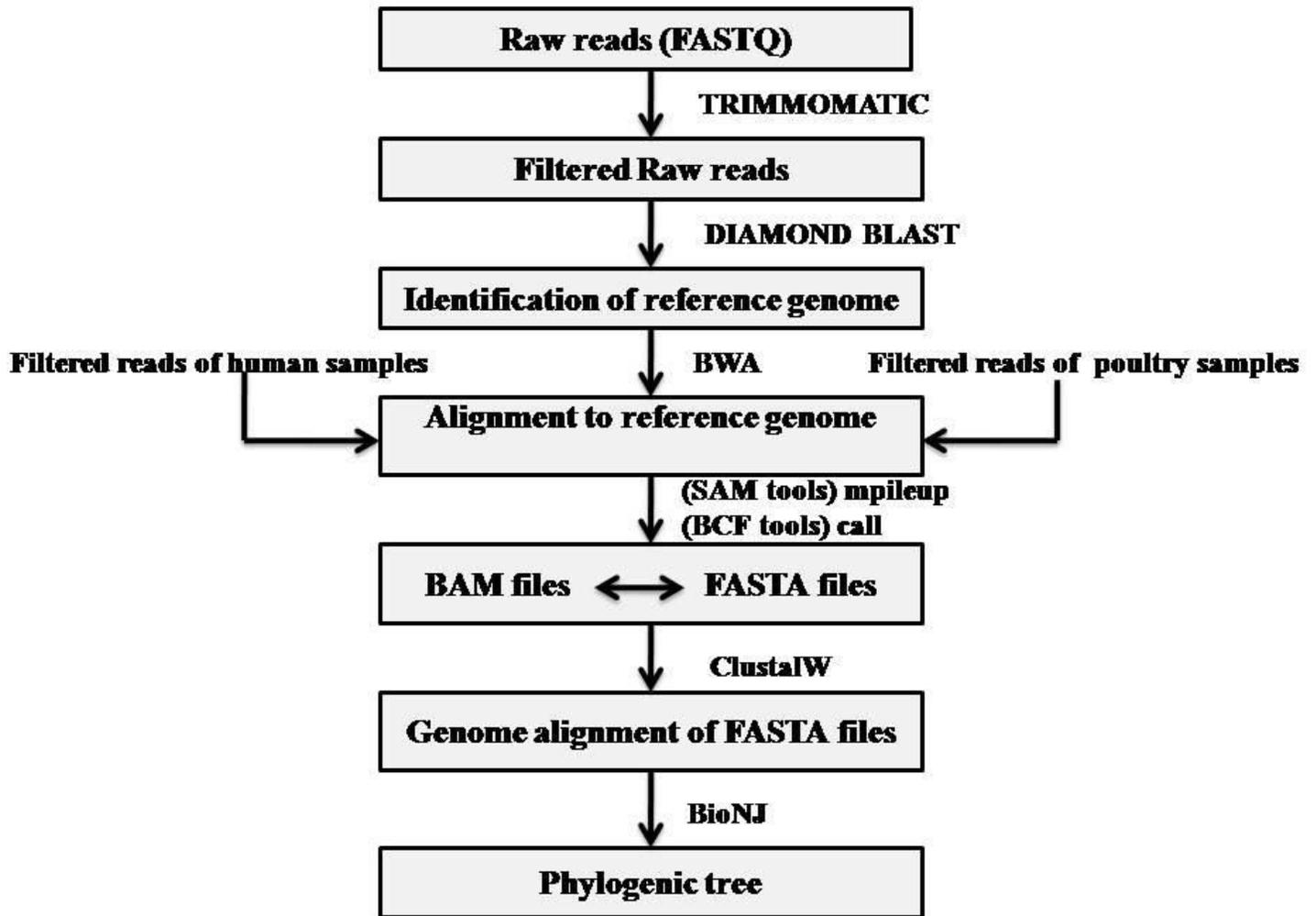Bioinformatics workflow of our custom data analysis pipeline

Figure 2

Bioinformatics workflow for the genetic analysis of HS and BC fecal samples with against the reference genome
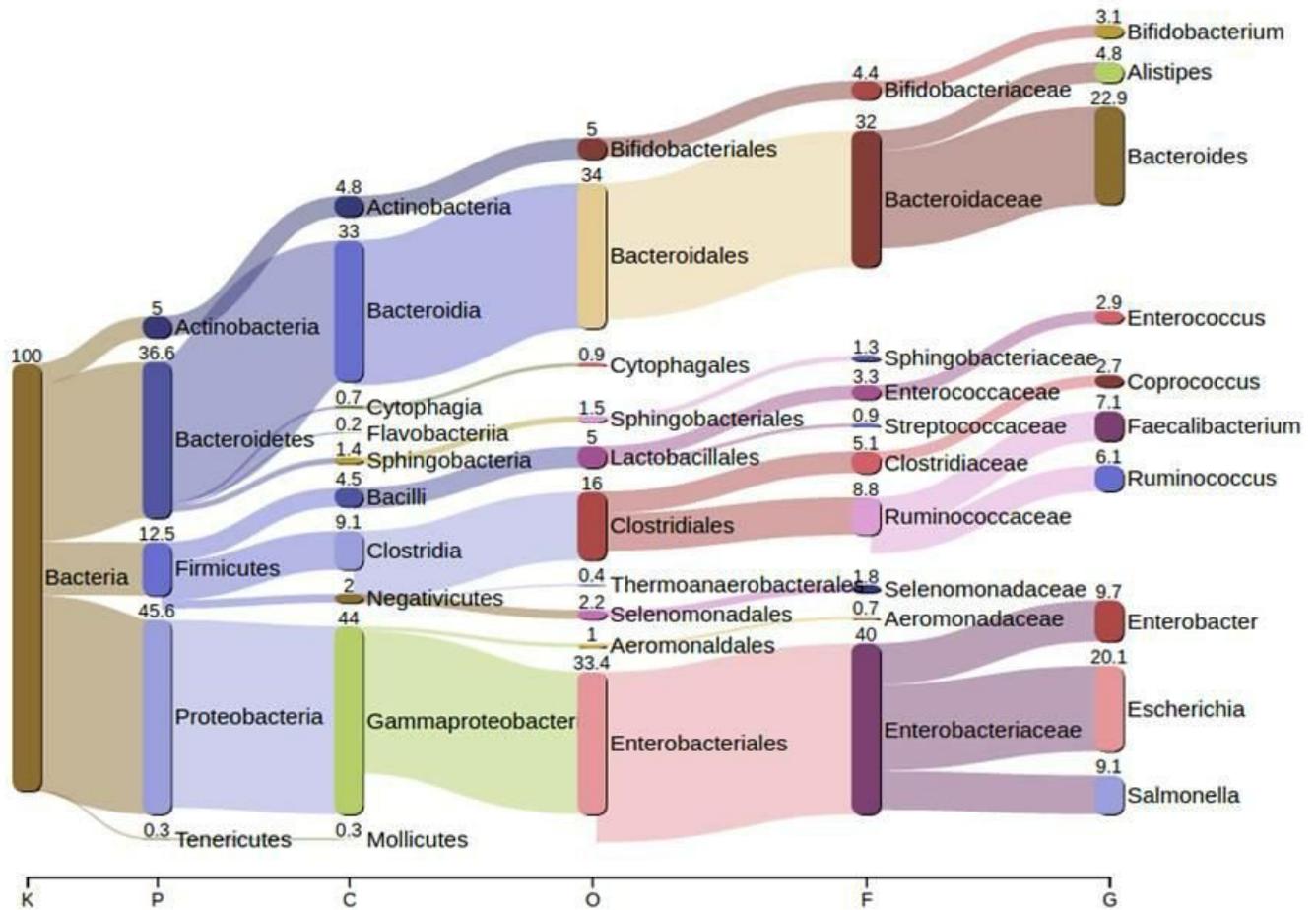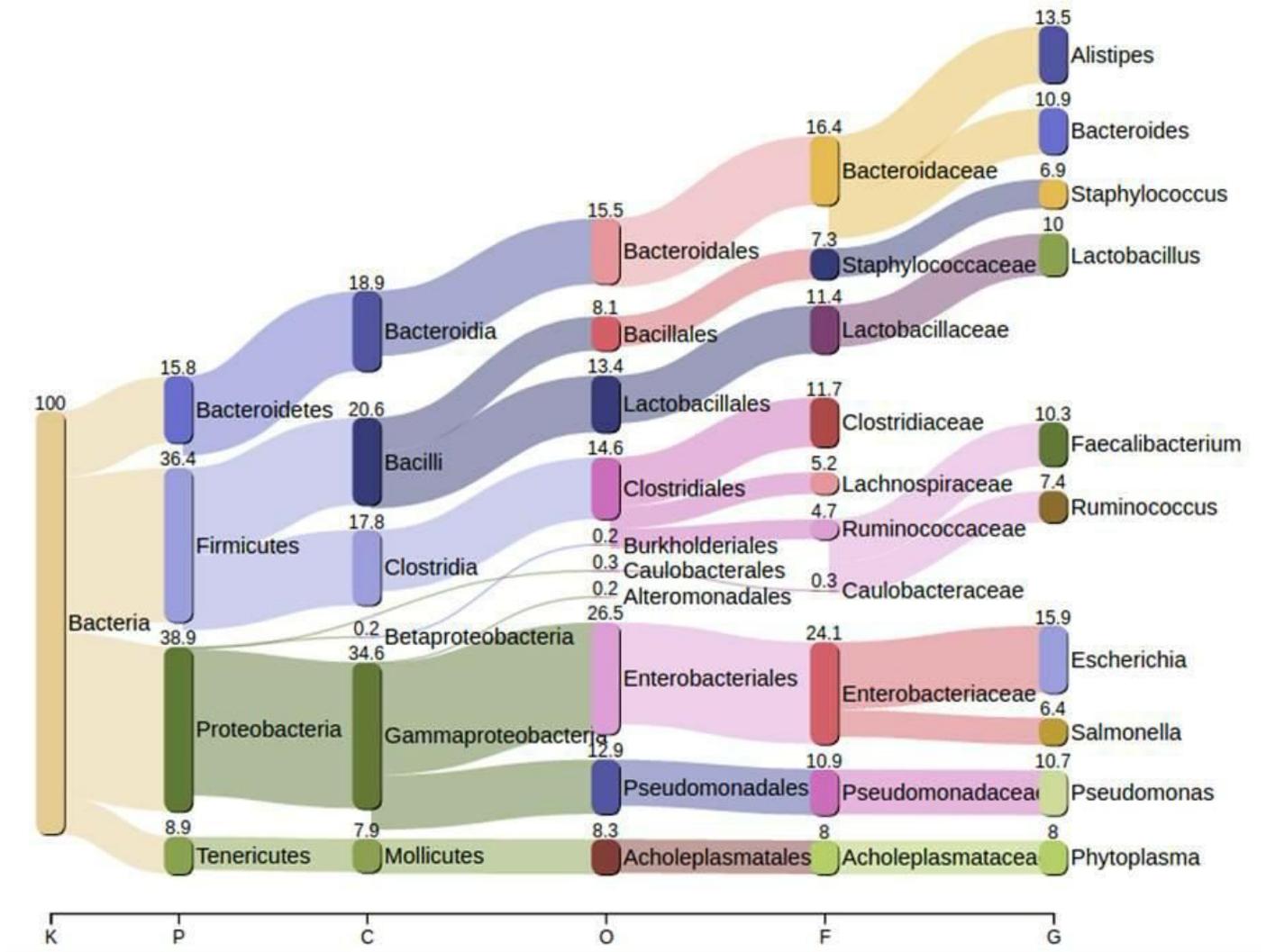
Figure 3

Taxonomic cladogram showing the bacterial diversity in HS microbiota at various levels using Pavian derived from NGS shotgun metagenomics. Different colored side bars in the Sankey diagram show the relative abundance of bacterial communities in human stool microbiota at various taxonomic levels including kingdom (K), phylum (P), class (C), order (O), family (F) and genus (G).

Figure 4

Taxonomic profiling of BC microbiota at various levels using Pavian derived from NGS shotgun metagenomics. Different colored side bars in the Sankey diagram show the relative abundance of bacterial communities in broilers chickens at various taxonomic levels including kingdom (K), phylum (P), class (C), order (O), family (F) and genus (G).
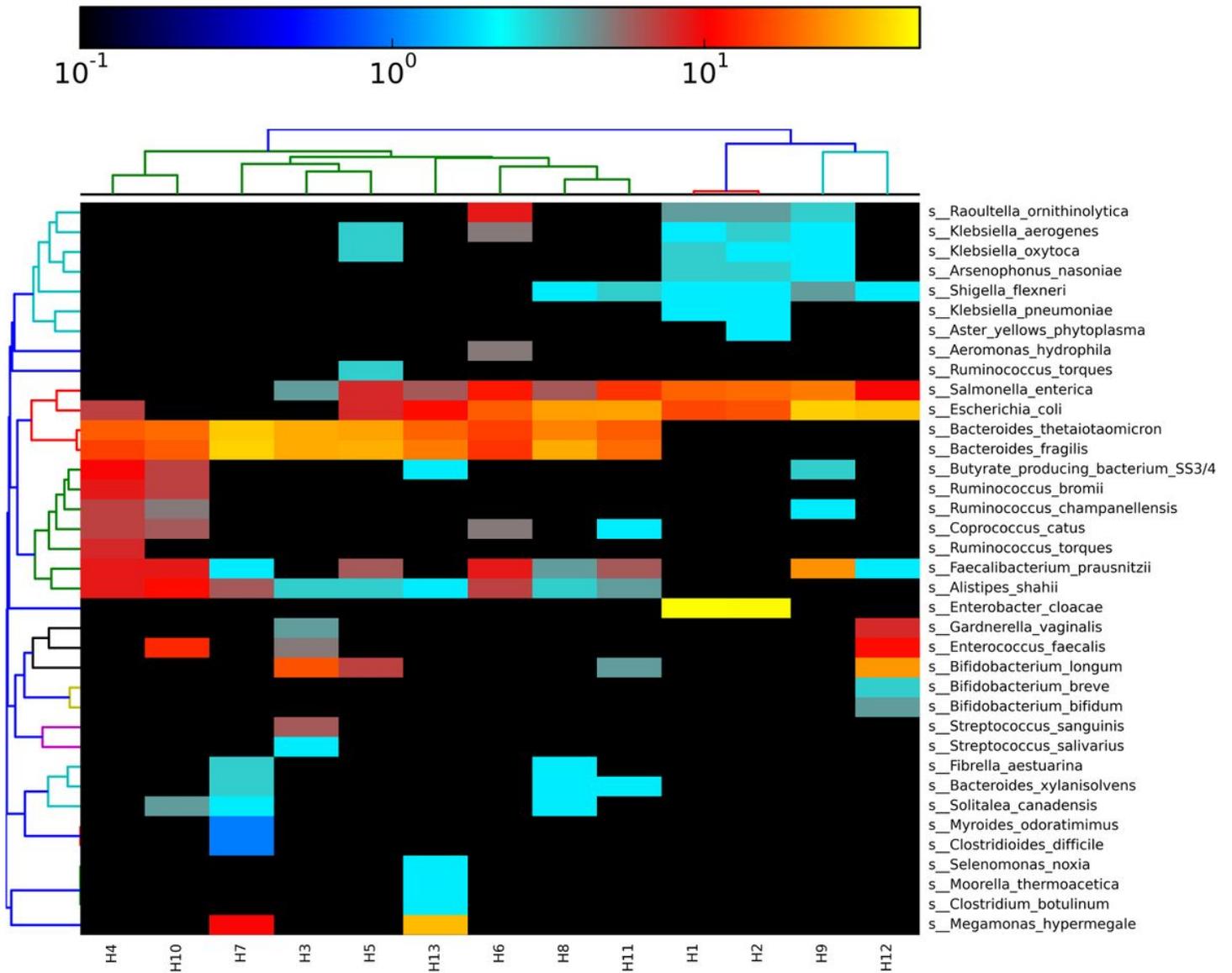
Figure 5

Heatmap generated through hclust2 showing the abundance of various bacterial species in HS microbiota derived from NGS shotgun metagenomics. Various bacterial species present within the HS microbiota are hierarchically clustered using Bray-Curtis dissimilarity. H1 to H13 represents various human stool samples. The relative abundance of various bacterial species is indicated in logarithmic values (base 10).
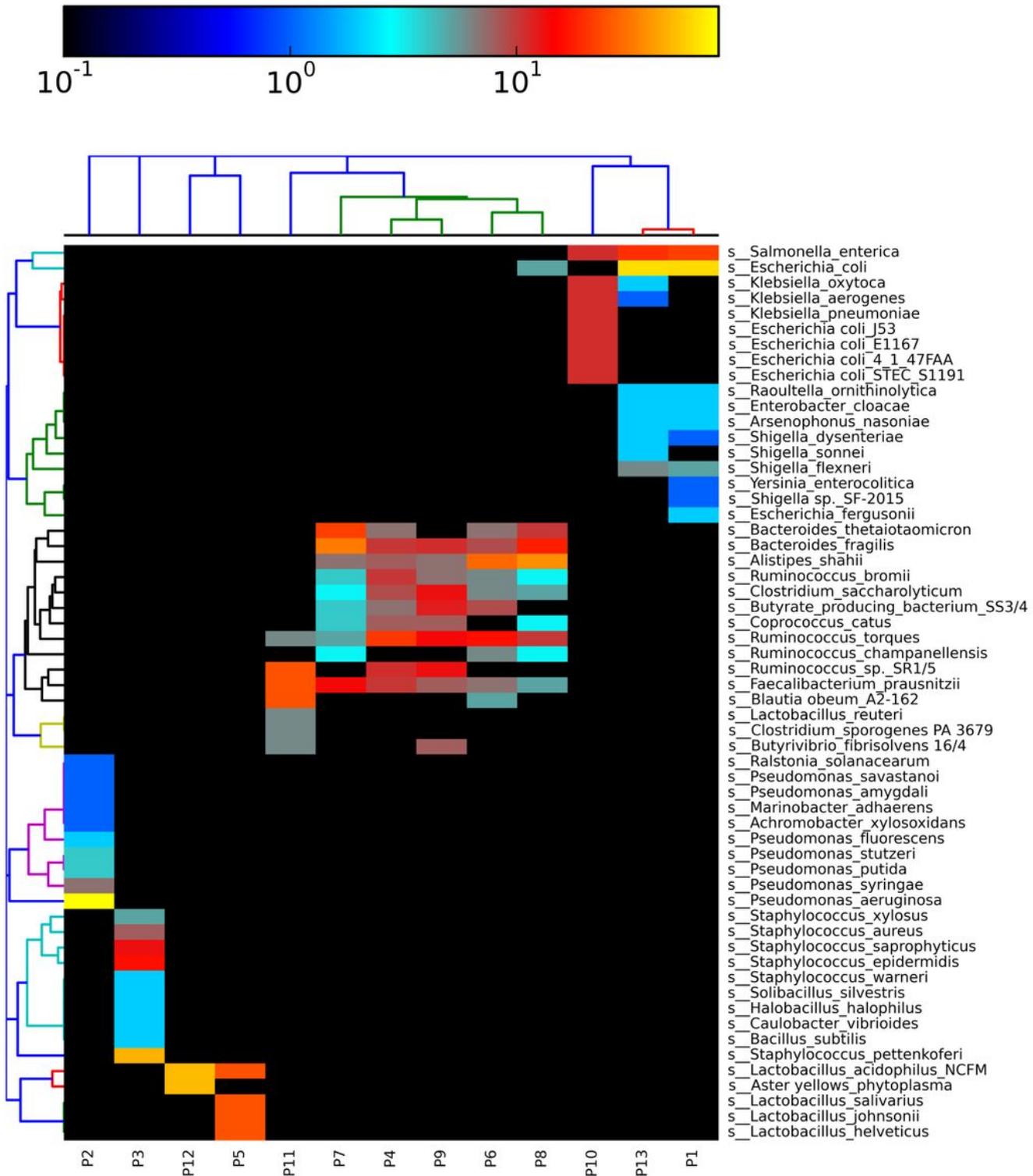
Figure 6

Heatmap generated through hclust2 showing the abundance of various bacterial species in BC microbiota derived from NGS shotgun metagenomics. Various bacterial species present within the human stool microbiota are hierarchically clusteredusing Bray-Curtis dissimilarity. Where P1 to P13 represents various broiler chicken samples i.e. poultry. The relative abundance of various bacterial species is indicated in logarithmic values (base 10).
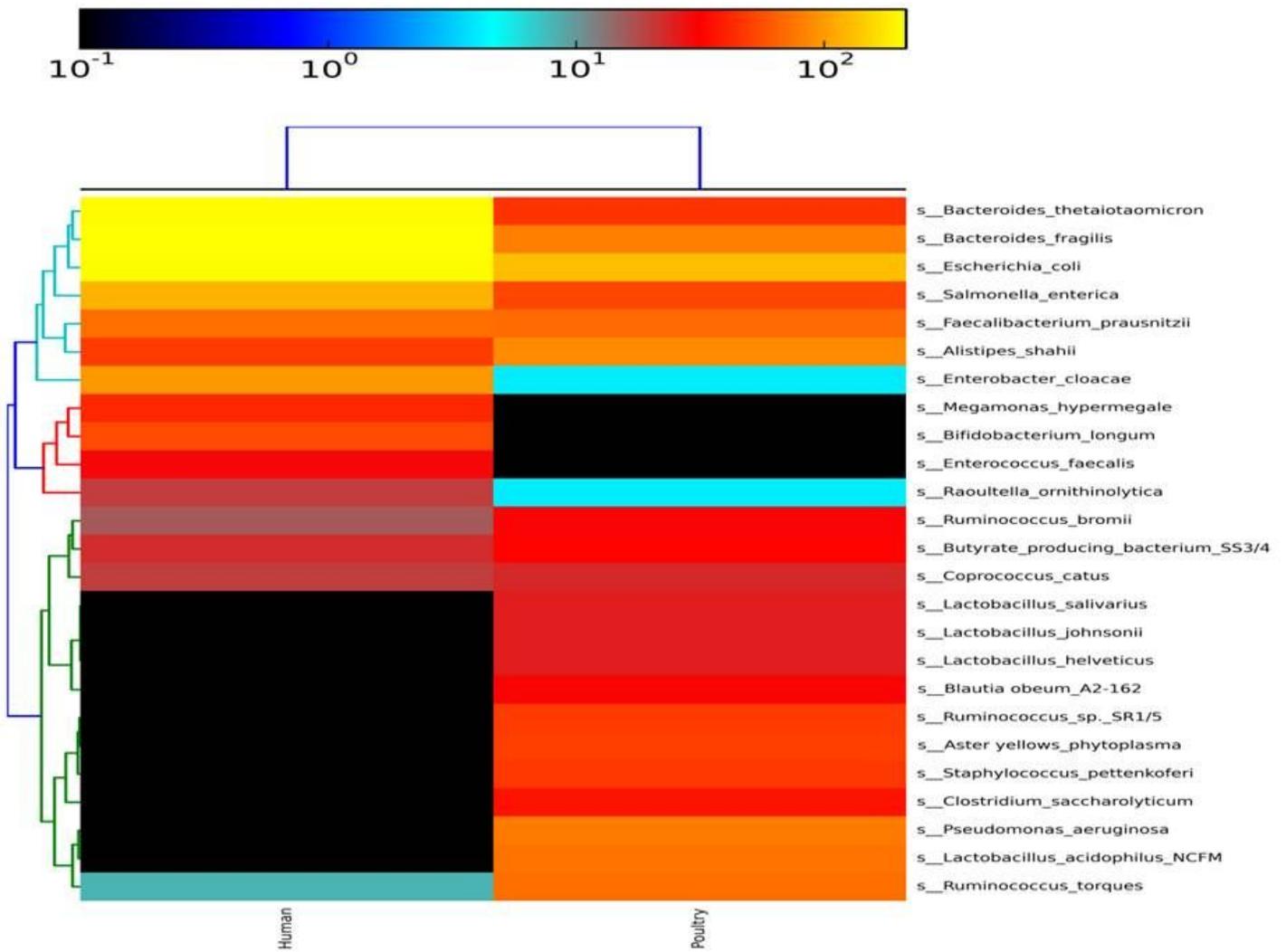
Figure 7

Combined heatmap generated through hclust2 showing the abundance of various bacterial species in HF and BC microbiota derived from NGS shotgun metagenomics. The diversity of bacterial species present within the human and poultry fecal microbiota are hierarchically clustered using Bray-Curtis dissimilarity. The relative abundance of various bacterial species is indicated by logarithmic values (base 10).
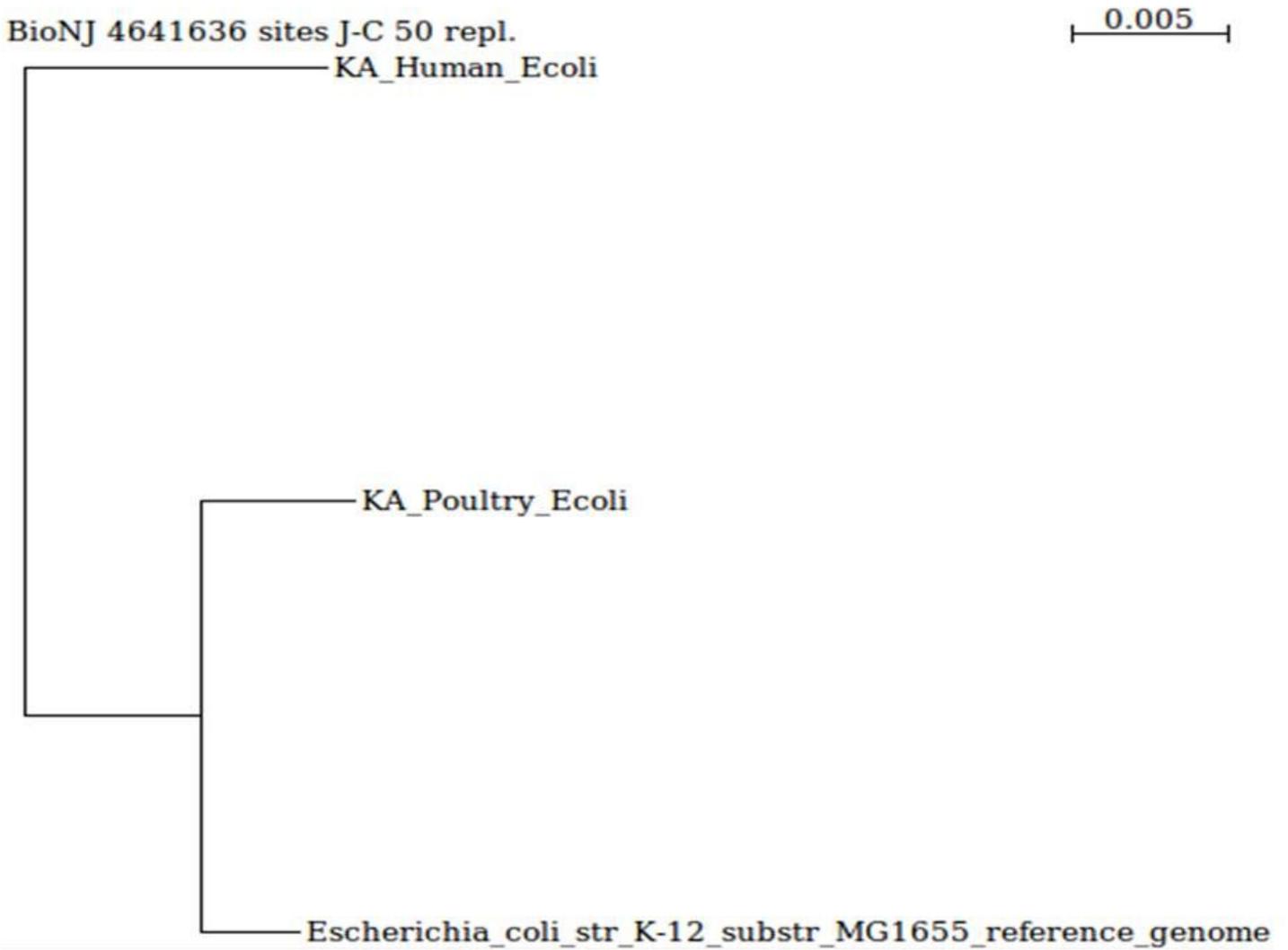
Figure 8

Phylogentic analysis of human and poultry E.coli against a reference strain E.coli str. K12 MG1655 reference genome. Where KA human E.coli and KA poultry E.coli describes E.coli from HS and BC fecal microbiota respectively.