16S rRNA Based Metagenomic Analysis of Bacterial Community Inhabiting the Gastrointestinal Tract of Domestic and Wild Chicken in Kohat

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ABSTRACT

Microbial communities are densely populated in chicken, a common poultry bird dominated by bacterial flora. The aim of the current study was to investigate the bacterial communities in Chickens' gastrointestinal tract (GIT) and their metagenomic comparison. GIT caecal contents were extracted from 2 healthy juvenile domestic and wild chickens of almost same weight and age were processed for Genomic DNA extraction by using QIAamp DNA Stool Mini Kit (QIAGEN, Germany). The DNA was amplified by using 16S rRNA gene primers and were subjected to cycle sequencing PCR by using 515F/806R. The illumina paired end FASTQ reads were analyzed with QIIME ver.2.2019.10 and SPSS ver. 22. A total of 193,537 High-quality 16S rRNA V4 (hyper variable region) gene amplicon sequences were obtained with 93,769 (domestic) and 99768 (wild chicken). The effective sequences were clustered into 1594 OTUs using a 97% sequence similarity value cut off then taxonomically delineated into 18 Phyla, 24 classes, 35 orders, 66 families, 133 genera, and 92 species. In general, the bacterial communities of both samples were dominated by Proteobacteria, Bacteriodetes and Firmicutes accounting for >95 % of all sequences but the relative abundances of these three phyla were quantitatively different amongst the domestic and wild chicken. The most abundant phyla were observed Proteobacteria accounted (56.69% and Bacteriodates (53.44%), classes were, Gammaproteobacteria (56.64%) and Bacteroidia (53.71%), orders were Cardiobacteriales (41.27%) and Bacteroidales (50.63%), families were wohlfahrtiimonadaceae (43.38%) and Bacteroidaceae (50.70%), genera were Ignatzschineria (21.11%) and Bacteroides (55.92%) and the species were *Myroides profundi* (43.69%) and *uncultured feedlot manure bacterium B1* (27.30%) in domestic and wild chicken respectively. The overall average similarities in phyla to species was recorded 25.27% in both wild and domestic chicken. The bacterial communities in the caeca of domestic chicken were more diverse in comparison to the wild chicken sample. The bacterial census created in this study might well identify gaps in knowledge on bacterial diversity in the poultry gastrointestinal tract.

Keywords: Domestic and wild chicken, Gastrointestinal tract, OTUs, Illumina, Metagenomics.

INTRODUCTION

Domestic chicken *Gallus gallus domesticus* is a common poultry bird that produces valuable meat and egg-based protein sources for humans. Chickens' gastrointestinal tract (GIT) is densely populated with diverse microbial communities (bacteria, fungi, archaea, protozoa and viruses) dominated by bacteria (Wei *et al*., 2013). The interactions between the host and the chicken the bacterial microbiome of GIT has been considered to play a vital role in nutrition, absorption, immunity development and prevention of the pathogenic and zoonotic bacterial colonization via competitive exclusion and the production of bacteriocin (Shang *et al*., 2018).

Bacterial colonization in GIT begins immediately after chick's hatch usually dominated by lactobacilli, Clostridiaceae, Streptococcus and Enterococcus (Ranjitkar *et al*., 2016). However several factors, such as the

environment, dietry supplementation, antibiotics therapy, race, genetics and age, can affect the intestinal microbiotic composition (Yegani, & Korver, 2008). Various approaches have characterized the chicken intestinal microbiota, from culture based data analysis to recent molecular techniques, with limited coverage and accuracy. Modern high-throughput (NGS) 16S rRNA sequencing approaches are capable of rapidly obtaining a total bacterial population census and metagenomics analysis quickly (Diaz-Sanchez *et al*., 2013).

The study of microbial diversity in avian is growing field throughout the world, with recent focus on commercially important species such as chicken, to enhance the poultry production and gut health (Stanley *et al*., 2014). However, the lack of sufficient knowledge on the bacterial diversity of poultry intestines in Kohat is considered one of the major knowledge gap. Therefore, the current study is designed to provide insight into the composition and diversity of bacterial community inhabiting the GIT of pure domestic chicken and wild chicken in Kohat zone Khyber Pakhtunkhwa using high throughput 16s RNA gene sequencing.

OBJECTIVES

1. To analyze the bacterial community composition in gastrointestinal tract (GIT) of domestic and wild chicken in Kohat.
2. Metagenomic comparison of bacterial community inhabiting GIT of domestic and wild chicken.

METHODOLOGY

GIT caecal contents were extracted from 2 healthy juvenile domestic and wild chicken of almost same weight and age. Genomic DNA was then extracted using QIAamp DNA Stool Mini Kit (QIAGEN, Germany) by following the manufacturer instructions and amplified, based on V4 hyper-variable region of 16S rRNA gene. Amplified DNA products were analyzed visually using 2 % (w/v) agarose gel electrophoresis (Xiao *et al*., 2016), while DNA quantity and purity was checked by nanodrop spectrophotometer. Lypholized PCR products were sequenced by Macrogen (south Korea). Illumina paired end FASTQ reads were analyzed with QIIME ver.2.2019.10 (Quantitative Insights into Microbial Ecology) manifest file import method and quality filtered using q2-dada2 denoising method (Bokulich *et al*., 2018). After discarding chimeric sequences the resulted Amplicon sequence variants (ASV) were taxonomically classified by q2-feature-classifier (Quast *et al*., 2012) trained on Silva 132_release 97% OTUs reference sequences (http://www.arb-silva.de/). The taxonomic composition of each sample at phylum, class, order and family taxonomic levels was generated with q2 taxa barplot method.

RESULTS

The objective of this study was to analyze the bacterial community composition and metagenomic comparison of bacterial communities inhabiting GIT of domestic and wild chicken using a naïve analysis of all the16S rRNA gene sequences. A total of 193,537 High-quality 16S rRNA V4 (hyper variable region) gene amplicon sequences were obtained of domestic and wild chicken gastrointestinal caecal origin (93,769 and 99768 respectively) from each sample. The effective sequences were clustered into 1594 OTUs using a 97% sequence similarity value cut off then taxonomically clustered into 18 Phyla, 24 classes, 35 orders, 66 families, 133 genera, and 92 species. A distinctive difference (74.73%) in bacterial communities was identified between GIT caecal sample of domestic and wild chicken. The bacterial communities in the caeca of domestic chicken were more diverse in comparison to the wild chicken sample. In general, the bacterial communities of both samples were dominated by Proteobacteria, Bacteriodetes and Firmicutes accounting for >95 % of all sequences but the relative abundances of these three phyla were quantitatively different amongst the domestic and wild chicken. Proteobacteria accounted (56% and 16.51%), *Bacteroidetes* (25.49% and 52.44%) and Firmicutes (16.58% and 29.90%) in domestic and wild chicken respectively. The most prevalent class was Gammaproteobacteria (56.64%), followed by Bacteroidia (25.50%) and Clostridia (14.87%). while Bacteroidia (53.71%) was the predominant class in wild chicken followed by Gammaproteobacteria (16.08%),

Bacilli (16.08%) and Clostridia (13.02%). At order level domestic chicken was dominated by Cardiobacteriales (41.27%) and wild was overwhelmed by Bacteroidales (50.63%). The most abundant 10 families (constituting 14%) accounted for 84% to 90% of the chicken bacterial flora dominated by Bacteroidaceae (50.70%). Ignatzschineria (21.11%) and Bacteroides (55.92%) were found most abundant genera in domestic and wild chicken caecal sample respectively (Table **1**, Figure **1**). Based on annotation of the sequence records, domestic and wild Chickens were shown to have different intestinal bacterias, sharing only 10.87% similarity at the species level. The most predominant species found in both the domestic and wild chicken sequence datasets were *uncultured feedlot manure bacterium B1*, *Myroides profundi*, *Lelliottia amnigena* and *swine effluent bacterium CHNDP1*. The top 10 bacterial species contributed 60.02% and 94.20 % of all the reads in domestic and wild chicken respectively.

CONCLUSION

In summary, this is the first description of the chicken bacterial communities using a metagenome sequence analysis in Khyber-Pakhtunkhwa Pakistan. Comparative metagenomic analyses identified apparent diverse differences in the structure of GIT bacterial communities between domestic and wild chicken. The GIT caecal bacterial communities of domestic chicken were more diverse in comparison to the wild chicken. This may be resulted from species-specific selective pressures, possibly dependent on behavioral, immune and metabolic characteristics. The main differences between bacterial communities were found to be related to different environment, nutrient as the diet, environment and specific endogenous factors affect the intestinal microbiota birds (Li *et al*., 2015).

Table 1. Overall diversity, richness and taxonomically Featured OTUs in numbers and % from phylum to species.

Index	Sample Chicken	Input	Phylum	Class	Order	Family	Genus	Specie
Diversity	Domestic (A)	Count	10	13	16	38	91	66
		%	(55.56)	(54.17)	(45.72)	(57.58)	(68.43)	(71.74)
	Wild (B)	Count	3	3	7	9	20	16
		%	(16.67)	(12.50)	(20)	(13.64)	(15.04)	(17.39)
	Common in A&B (C)	Count	5	8	12	19	22	10
		%	(27.78)	(33.33)	(34.29)	(28.79)	(16.55)	(10.87)
	Total (A+B+C)100%		18	24	35	66	133	92
Richnes of ASVs	Domestic (A)	93,769	93,684	93,658	93,129	88,600	59,204	44,886
		48.45%	(48.40)	(48.39)	(48.11)	(45.77)	(30.59)	(23.19)
	Wild (B)	99,768	99,727	99,217	98,548	96,338	87,339	19,053
		51.54%	(51.52)	(51.26)	(50.91)	(49.77)	(45.12)	(9.84)
	Total (A +B)	193,537	193,411	192,206	191,677	184,938	146,573	63,939
		100%	(99.92)	(99.65)	(99.02)	(95.54)	(75.71)	(33.03)
OTUs featured	Both Domestic & wild	1,594	1,578	1,572	1,527	1,027	750	548
		100%	(98.99)	(98.61)	(95.79)	(64.42)	(47.05)	(34.37)

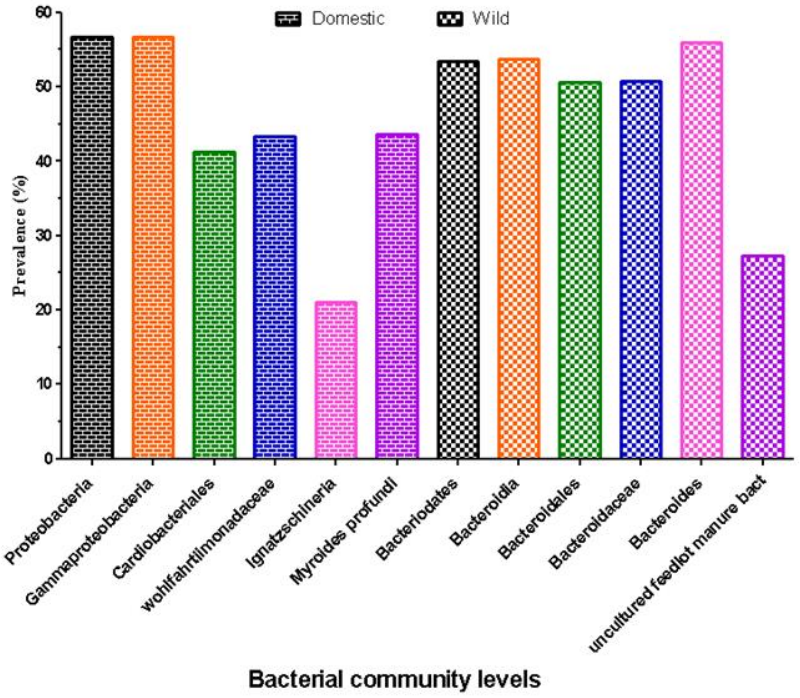

Figure 1. Overall abundant bacterial community levels (%) in GIT caecal samples of domestic and wild chicken.

RECOMMENDATIONS

The bacterial census created in this study might well identify gaps in knowledge on bacterial diversity in the poultry gastrointestinal tract to establish a baseline framework for future research on chicken microbiology, and development of analytic tools, as well make an original contribution to the artificial rearing of these birds.

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