Microbial Pathogenesis 121 (2018) 210–217

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Microbial shifts in the swine nasal microbiota in response to parenteral antimicrobial administration

Mohamed Zeineldin[a,b], Brian Aldridge[a], Benjamin Blair[a], Katherine Kancer[a], James Lowe[a,*]

[a] Integrated Food Animal Management Systems, Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, USA

[b] Department of Animal Medicine, College of Veterinary Medicine, Benha University, Egypt

ARTICLE INFO

Keywords:
Antimicrobials
Swine
Nasal
Microbiota
16S rRNA gene

ABSTRACT

The continuous administration of antimicrobials in swine production has been widely criticized with the increase of antimicrobial-resistant bacteria and dysbiosis of the beneficial microbial communities. While an increasing number of studies investigate the effects of antimicrobial administration on swine gastrointestinal microbiota biodiversity, the impact of their use on the composition and diversity of nasal microbial communities has not been widely explored. The objective of this study was to characterize the short-term impact of different parenteral antibiotics administration on the composition and diversity of nasal microbial communities in growing pigs. Five antimicrobial treatment groups, each consisting of four, eight-week old piglets, were administered one of the antimicrobials; Ceftiofur Crystalline free acid (CCFA), Ceftiofur hydrochloride (CHC), Tulathromycin (TUL), Oxytetracycline (OTC), and Procaine Penicillin G (PPG) at label dose and route. Individual deep nasal swabs were collected immediately before antimicrobial administration (control = day 0), and again on days 1, 3, 7, and 14 after dosing. The nasal microbiota across all the samples were dominated by *Firmicutes*, *proteobacteria* and *Bacteroidetes*. While, the predominant bacterial genera were *Moraxella*, *Clostridium* and *Streptococcus*. Linear discriminant analysis, showed a pronounced, antimicrobial-dependent microbial shift in the composition of nasal microbiota and over time from day 0. By day 14, the nasal microbial compositions of the groups receiving CCFA and OTC had returned to a distribution that closely resembled that observed on day 0. In contrast, pigs that received CHC, TUL and PPG appeared to deviate away from the day 0 composition by day 14. Based on our results, it appears that the impact of parenteral antibiotics on the swine nasal microbiota is variable and has a considerable impact in modulating the nasal microbiota structure. Our results will aid in developing alternative strategies for antibiotics to improve swine health and consequently production.

1. Introduction

Antimicrobials have been used for over 60 years at all stages of swine production for disease prevention and growth promotion [1] [2]. The use of antimicrobials in livestock production has been widely criticized with the increase of drug resistant microbes and residual antibiotics in the animal tissue [3]. Negative public perception on the use of antimicrobials in animal agriculture, as well as recent legislative changes in select European countries, have led US veterinarians and producers to foresee a future where antimicrobial use will be more limited [4]. In order to overcome these challenges, production systems must adapt by reducing the overall need for antimicrobials [5]. A key step in this process will be to shift from pathogen focused to host focused approaches in controlling disease. This entails optimizing host defenses against infectious disease, primarily through improving local innate and adaptive immune defenses.

While the composition and functional role of nasal microbiota in pigs have been a significant research topic for decades, most of these previous studies were restricted by their dependence on traditional culture-dependent methods, which provides a relatively narrow picture of the complexity of these microbial populations [6] [7] [8]. The advancements in bioinformatics and next generation sequencing technologies targeting the 16S rRNA gene have revealed a greater diversity of microbial communities in respiratory tract, and helped researchers in describing the complex interplay between the host and microbial ecosystems [9] [10]. Recent research studies suggest that nasal microbiota is comprised of a diverse and complex microbial population that influences many immunological processes in the host and contribute to

* Corresponding author. Integrated Food Animal Management Systems, 2938 VMBSB, 2001 South Lincoln Avenue, Urbana, IL 61802, USA
E-mail addresses: zeineldn@illinois.edu (M. Zeineldin), Ba311@illinois.edu (B. Aldridge), bblair2@illinois.edu (B. Blair), kancer1@illinois.edu (K. Kancer), jlowe@illinois.edu (J. Lowe).

https://doi.org/10.1016/j.micpath.2018.05.028
Received 24 February 2018; Received in revised form 28 April 2018; Accepted 18 May 2018
Available online 24 May 2018
0882-4010/ Published by Elsevier Ltd.

development of respiratory disease [11] [12].

The composition of the healthy nasal microbiota is dynamic, and can be disturbed by changes in diet, age, administration of antimicrobial agents, and many other factors [12]. The impact of antimicrobial administration on the composition of microbiota depends, not only on the chemical structure of the antimicrobial agent, but also on the route of administration, duration, and dose [13]. It has been shown that antimicrobials can cause a temporal decline in gastrointestinal microbial diversity [14]. These changes can be detected as early as 24 h after antimicrobial administration, with profound changes still evident at 7 days [15] [16], and only a partial recovery of the population stability by 30–40 days after treatment [17]. While some investigators have demonstrated some resilience in return to a pretreatment community structure, others have shown that it may take years for the taxonomic composition to completely recover [18]. Consequently, the objective of this study was to use 16S rRNA Illumina-based sequencing to characterize the impact of parenteral antimicrobial administration (CCFA, CHC, TUL, OTC, and PPG) on the composition and diversity of nasal microbial communities in a population of growing pigs maintained in a commercial research swine operation. We also sampled the treated pigs at different time-points post treatment to determine if the microbiota recovers to the pretreatment status.

2. Materials and methods

2.1. Experimental design and sample collection

Five antimicrobial treatment groups each consisting of four, eight-week-old piglets housed in a single facility in the Midwest were used in this study under written consent [15]. All pigs, which were from a single source and were the only pigs on the site, had been housed together since weaning. Pens were separated by an open pen so there was no nose-to-nose contact between the pens. All pigs were fed the same diet and had not received any antibiotics in the 7 weeks prior to enrollment. The pigs in each group (n = 4) received a different antimicrobial regime [CCFA group - Ceftiofur Crystalline free acid (5.0 mg /kg IM); CHC group - Ceftiofur hydrochloride (5 mg/kg IM); TUL group - Tulathromycin (2.5 mg/kg IM); OTC group - Oxytetracycline (9 mg/lb IM); PPG group -Procaine Penicillin G (15,000 units/lb IM)]. The dosages of antibiotics and route of administration were based on manufacturer label instruction except for PPG, which was given at accepted dosage levels currently in US swine herds [19]. Deep nasal swabs were taken from each piglet at different time points, (day 0) immediately before antibiotic administration and again on days 1, 3, 7, and 14. Once collected, all samples were placed on dry ice, transported to the lab and stored at −80°C until further processing and analysis. All animal protocols were approved by Illinois University Institutional Animal Care and Use Committee (IACUC).

2.2. DNA extraction

Nasal swabs were processed for total DNA extraction using PowerFecal® DNA Isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) following manufacturer's instructions. For each sample, DNA concentration and purity were evaluated by optical density using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE, USA) at wavelengths of 230, 260 and 280 nm and the DNA integrity was evaluated by agarose gel electrophoresis (Bio-Rad Laboratories, Inc, Hercules, CA, USA).

2.3. DNA amplification and illumina sequencing

Extracted DNA samples from each nasal swab was subjected to Fluidigm Access Array Amplification (Fluidigm Corporation, South San Francisco, CA, USA) of the V1-V3 hyper-variable region of the 16S rRNA gene. Before amplification, all DNA samples were measured on a Qubit fluorometer (Life technologies, Grand Island, NY, USA) using the High Sensitivity DNA Kit (Roche, Indianapolis, IN, USA) and 20x Access Array loading reagent according to Fluidigm protocols as mentioned in [20] [21].

Table 1
Access Array cycling program without imaging (Fluidigm Biomark HD PCR machine) for amplifying the primer/sample combinations.

PCR Stages	Number of Cycles
50 °C 2 min	1
70 °C 20 min	1
95 °C 10 min	1
• 95 °C 15 s	
• 55 °C 30 s	
72 °C 1 min	10
• 95 °C 15 s	
• 80 °C 30 s	
• 60 °C 30 s	
72 °C 1 min	2
• 95 °C 15 s	
• 55 °C 30 s	
72° 1 min	8
• 95 °C 15 s	
• 80 °C 30 s	
• 60 °C 30 s	
72 °C 1 min	2
• 95 °C 15 s	
• 55 °C 30 s	
72 °C 1 min	8
• 95 °C 15 s	
• 80 °C 30 s	
• 60 °C 30 s	
72 °C 1 min	5

The primers F28-2-for ACACTGACGACATGGTTCTACA and R519-2-rev TACGGTAGCAGAGACTTGGTCT were designed for the amplification process. CS1 forward and CS2 reverse tag with unique eight-base sequence barcode were added to each sample according to the Fluidigm protocol instructions (Fluidigm Corporation, South San Francisco, CA). Following the loading stage, the IFC fluidigm specific plate (Fluidigm Corporation, South San Francisco, CA) was loaded on the Fluidigm Biomark HD PCR machine (Fluidigm Corporation, South San Francisco, CA), and samples were amplified using the following Access Array cycling program without imaging (Table 1). The final harvested product was then quantified on a Qubit fluorometer the quality of the amplicons regions was assessed using a Fragment Analyzer (Advanced Analytics, Ames, IA) to confirm amplicon regions and sizes. DNA samples were then pooled in equal amounts according to product concentration. The pooled samples were then size selected on a 2% agarose E-gel (Life technologies, Grand Island, NY) and extracted using Qiagen gel purification kit (Qiagen, Valencia, CA). Cleaned size-selected pooled products were run on an Agilent Bioanalyzer to confirm appropriate profile and average size. The final pooled Fluidigm libraries were transferred to the DNA Services lab at the W. M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign for Illumina sequencing. The Illumina MiSeq platform (Illumina, San Diego, CA, USA) was used to sequence the V1- V3 region of the 16S rRNA gene according to the Illumina instructions. The libraries were sequenced from both ends of the molecules to a total read length of 300nt from each end according to the Illumina instructions (Illumina, San Diego, CA).

2.4. Sequence data processing and statistical analysis

The Illumina base call (bcl) files generated from the sequence run were demultiplexed and compressed using bcl2fastq 1.8.4 (Illumina, CA). A secondary pipeline in bcl2fastq 1.8.4 was used to report the number of sequence reads per sample per library. The 16S rRNA gene