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Microbial ecology, bacterial pathogens, and antibiotic resistant genes in swine manure wastewater as influenced by three swine management systems☆

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ARTICLE INFO

Article history:
Received 26 October 2013
Received in revised form
4 March 2014
Accepted 9 March 2014
Available online 19 March 2014

Keywords:
Confined animal feeding operation (CAFO)
Antibiotic resistance
Campylobacter
Salmonella
Swine
Lagoon wastewater

ABSTRACT

The environmental influence of farm management in concentrated animal feeding operations (CAFO) can yield vast changes to the microbial biota and ecological structure of both the pig and waste manure lagoon wastewater. While some of these changes may not be negative, it is possible that CAFOs can enrich antibiotic resistant bacteria or pathogens based on farm type, thereby influencing the impact imparted by the land application of its respective wastewater. The purpose of this study was to measure the microbial constituents of swine-sow, -nursery, and -finisher farm manure lagoon wastewater and determine the changes induced by farm management. A total of 37 farms were visited in the Mid-South USA and analyzed for the genes 16S rRNA, *spaQ* (*Salmonella* spp.), Camp-16S (*Campylobacter* spp.), *tetA*, *tetB*, *ermF*, *ermA*, *mecA*, and *intI* using quantitative PCR. Additionally, 16S rRNA sequence libraries were created. Overall, it appeared that finisher farms were significantly different from nursery and sow farms in nearly all genes measured and in 16S rRNA clone libraries. Nearly all antibiotic resistance genes were detected in all farms. Interestingly, the *mecA* resistance gene (e.g. methicillin resistant *Staphylococcus aureus*) was below detection limits on most farms, and decreased as the pigs aged. Finisher farms generally had fewer antibiotic resistance genes, which corroborated previous phenotypic data; additionally, finisher farms produced a less diverse 16S rRNA sequence library. Comparisons of Camp-16S and *spaQ* GU (genomic unit) values to previous culture data demonstrated ratios from 10 to 10,000:1 depending on farm type, indicating viable but not cultivatable bacteria were dominant. The current study indicated that swine farm management schemes positively and negatively affect microbial and antibiotic resistant populations in CAFO wastewater which has future "downstream" implications from both an environmental and public health perspective.

Published by Elsevier Ltd.

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http://dx.doi.org/10.1016/j.watres.2014.03.017
0043-1354/Published by Elsevier Ltd.

1. Introduction

Ecological adaptation, in any environment, is necessary for survival. Many factors influence bacterial adaptation within concentrated animal feeding operations (CAFOs); particularly, animal age and type, management (feeding and antibiotic use), and CAFO house design. Within a single industry, it is expected that animal age and animal management yield the most influence. Swine CAFOs can be separated into three stages or types based on age, each with their own animal and waste management: 1) sow (breeding, gestation, farrowing); 2) nursery (21 d–18 kg feeders); and 3) finisher farms (feeders to 113 kg) (McLaughlin et al., 2009). At each stage, animal management, including antibiotics, is carefully employed to sustain growth or treat infection (Sengelov et al., 2003; Rajic et al., 2006). Typically, swine liquid manure (e.g. wastewater) is land applied, which is essential to farm sustainability, regardless of farm stage (McLaughlin et al., 2009). Thus, each operation imposes its own selective pressures on gut microbiota and manure microbial populations; changes to antibiotic resistance, pathogens, and microbial ecology can serve as indicators, shedding light on agriculture's role in public and environmental health.

Some swine operators are shifting to focus on one developmental stage. This shift is largely dependent on market demands, centralized distribution, or environmental regulation. Nutrients, pathogens, and antibiotic resistance can be influenced by swine farm type (McLaughlin et al., 2009; Brooks and McLaughlin, 2009). Brooks and McLaughlin (2009) demonstrated a marked increase in antibiotic resistance from sow and nursery farms compared to finisher farms. A broad-range of antibiotics are administered on a large-scale basis, often in feed and water, throughout the pig rearing process, with a focus at early stages (Jindal et al., 2006; Rajic et al., 2006). Previous research only considered cultivated antibiotic resistant and pathogenic bacteria (Leung and Topp, 2001, Sengelov et al., 2003, Chinivasagam et al., 2004; Binh et al., 2008), though few studies measured genotypic resistance (Barkovskii et al., 2012; Chen et al., 2010). It is well known, that cultivation captures ~0.1% of bacteria; thus, potential antibiotic resistance, from both pathogenic and commensal bacteria, are uncounted. Quantitative polymerase chain reaction (qPCR), which is culture independent, overcomes this deficiency, yielding a more conservative quantification of microbial risks, which ultimately affects microbial risk assessment (Brooks et al., 2012).

Swine manure wastewater research recently has incorporated 16S rRNA fingerprinting, sequence libraries, and qPCR. Cotta et al. (2003), using a combination of culture and 16S rRNA gene sequencing, determined that lagoon wastewater was dominated by *Clostridium*, *Enterococcus*, and *Bacteroides*. Hog management and lagoon physicochemistry have led to temporal shifts in the microbial population (Cook et al., 2010; Lovanh et al., 2009). However, these studies focused on one swine farm type and offered no comparison based on farm type.

Therefore, the purpose of this study is to determine the effect of three different farm types: sow, nursery, and finisher farm management on select microbial populations of manure lagoon wastewater using culture-independent methodologies. The effect of farm management was quantified and qualified by targeting total eubacterial (i.e. 16S rRNA), antibiotic resistant, and bacterial pathogenic populations.

2. Materials and methods

2.1. Sample collection

Samples were collected in conjunction with previous studies (Brooks and McLaughlin, 2009; McLaughlin et al., 2009). Briefly, samples were collected from 37 anaerobic swine manure lagoons located in the Southeastern United States, from three different farm types, comprised of 17 sow, 10 nursery, and 10 finisher farms. Samples were collected at six locations per lagoon (three each on opposite lagoon sides) in sterile 250 ml polypropylene bottles using a modified PVC floatation float (McLaughlin et al., 2014). Sample aliquots were immediately frozen at −20 °C overnight and transferred to −65 °C for permanent storage.

2.2. DNA extraction

Frozen samples were thawed in a 25 °C waterbath prior to DNA extraction. Samples were processed by compositing 5 ml from each of 6 sub-samples, per lagoon, followed by microbial DNA extraction using a modified procedure employing the Mobio Power Soil DNA Extraction Kit (Mobio Laboratories, Inc.; Carlsbad, CA) and the Qiagen Qiamp DNA Stool kit (Qiagen; Valencia, CA). Briefly, the Power Soil kit was modified by removing lysis buffer from the bead beating tubes and replaced with 2 ml of the composited sample. The sample was then centrifuged at 20,000× *g* for 3 min and repeated three times, with interval ice steps. The supernatant was discarded at the final step, followed by addition of 450 μl of Qiagen ASL buffer and mixed by vortex. The entire volume was placed in a Fast Prep FP120 (Qbiogene, Inc.; Carlsbad, CA) at speed setting 5.0 for 20 s, and repeated three times with interval ice steps. Following shaking, a 20 μl lysozyme solution (65 mg ml^{-1}) was added to the mixture and incubated at 37 °C for 20 min, followed by 99 °C in a dry heat block for 10 min. The heated solution was vortexed for 15 s and centrifuged at 20,000× *g* for 1 min. The Qiagen Stool DNA extraction kit was then followed beginning with step 6 of the manufacturer's protocol.

2.3. PCR analysis

The resulting DNA was assayed for the presence of eubacterial, pathogenic, and antibacterial resistance genes: 16S rRNA (total eubacteria), *spaQ* (*Salmonella* spp.), Camp-16S (*Campylobacter*), *tetA*, *tetB*, *intI*, *ermA*, *ermF*, and *mecA* using qPCR. Primer pairs used in the study were as follows: 16S rRNA-16Sfor/rev (Nadkarni et al., 2002), *Salmonella* spp.-spaQF/R (Kurowski et al., 2002), *Campylobacter* spp.-campF2/R2 (Lund and Madsen, 2006), tetracycline resistance-tetAF/R and tetBF/R (Fan et al., 2007), class I integron-intIF/R (Hardwick et al., 2008), erythromycin resistance-ermAF/R and ermFF/R (Chen et al.,2010), and methicillin resistance-mecAF/R (Sabet et al.,