BACKGROUND LEVELS AND DISSEMINATION PATHWAYS OF CLINICALLY RELEVANT ANTIBIOTIC RESISTANCE GENES IN PRISTINE AND AGRICULTURAL ENVIRONMENTS MONITORED OVER A CROP-GROWING SEASON.

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INTRODUCTION

Antimicrobial resistance severely hampers the treatment of infectious diseases in humans and animals and poses a substantial threat to Public Health and the global economy. Many antibiotic resistance genes (ARGs) that inactivate clinically important antimicrobials appear to originate from environmental reservoirs like soil or water compartments. Along the food/feed chain, agricultural soils constitute a source and sink for environmentally-borne and anthropogenically-introduced ARGs. Antibiotic resistant bacteria and ARGs from manure generated in animal husbandry interact with endogenously present bacteria and ARGs in agricultural fields and can re-enter the food/feed chain via plant-derived feed contaminated with ARGs.

ARG concentrations and dissemination pathways were monitored for a complete crop growth season in an open-air testing range consisting of pig farms and associated crop fields that were fertilised artificially or with locally generated manure. The results were compared with naturally occurring ARG concentrations in pristine soils under low anthropogenic exposure (protected wetlands, alpine area), allowing an evidence-based identification of high-risk compartments for AMR dissemination.

METHODOLOGY

Total DNA was isolated in triplicate from soils collected periodically from maize fields with artificial and organic fertilisation located in the Hydrology Open Air Laboratory (HOAL, Petzenkirchen, Austria) with the Powersoil DNA isolation kit (Qiagen). Total soil DNA from non-agricultural comparator areas was collected from pristine alpine regions and protected wetlands in a national park, and from inner-city park areas and boulevards under increasing anthropogenic exposure. Pig manure and faeces were also analysed. ARG relative and absolute abundances per gram of soil (dry-weight) were determined using TaqMan qPCR (Ingenetix) on the LightCycler 480 (Roche) for the following targets: sul1, ermB, vanA, tet(W), blaTEM-1, aph(3')-IIa, and aph(3')-IIIa, which inactivate sulfonamides, macrolides, glycopeptides, tetracyclines, penicillins and aminoglycosides, respectively. 16S was used to calculate the bacterial cell number and relative ARG abundances. Bacterial biodiversity was

determined by 16S amplicon sequencing (Illumina MiSeq). Soil samples were characterised for common soil parameters and heavy metals according to appropriate ÖNORM guidelines and for the presence of antibiotics.

RESULTS

The concentrations of certain ARGs significantly increased one day after manure application in exposed agricultural soils (baseline/peak/after harvest; copies per gram (dry weight): sul1 (10E6/10E7/10E6), ermB (10E4/10E7/10E4), tet(W) (10E5/10E7/10E6), aph(3')-IIIa (10E5/10E6/10E4) which gradually returned to the baseline levels present at the onset of the experiment over the crop growing season. A significant manure-associated peak for blaTEM-1 (10E3/10E4/10E3) could be observed only one week after exposure.

VanA concentrations were not influenced by manure and decreased over the whole observation period (10E5-10E4). Aph(3')-IIa was only sporadically detected, usually at low concentrations (10E2).

The forest closely associated with the fields showed more than 10E5 copies of tet(W), corresponding to the baseline levels observed in the fields andtested pristine areas. Pristine protected national park wetland and alpine soil samples showed distinctly lower sul1 concentrations compared to the background loads observed in manured agricultural fields but similar concentrations to the non-manured field over the whole monitoring period. Similar observations were made as regards tet(W) and ermB.

DISCUSSION

Challenging agricultural fields with ARG-containing manure led to an increase in the abundances of certain soil-borne ARGs. These concentrations decreased over a period of approx. 10 months to the baseline levels, indicating that these soils have a high capacity for resilience. This observation suggests that the ARG dissemination route via freshly introduced manure-derived gut bacteria is not capable of permanently establishing exogenous ARB and/or ARGs in exposed soil microbial communities. Tet(W) and sul1 are omnipresent in agricultural and “pristine” soils from protected wetlands and alpine regions under low anthropogenic pressure. Their naturally occurring background concentrations are comparable with the baseline abundances observed in non-manured fields. Antibiotics could not be detected in the soils. Other mechanisms must be responsible for these notable naturally occurring ARG background loads (10E4 to 10E5 copies/g). The collected data indicate that roughly 1 in 10 000 bacteria might be a carrier of at least one of these clinically relevant ARGs in soils regardless of whether they originate from pristine or anthropogenically-influenced environments such as agricultural fields.