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Clinical Microbiology 2018 : Comparison of Bacterial Community Structure and Diversity in Traditional Gold Mining Waste Disposal Site and Rice Field by Using a Meta barcoding Approach- Fatimawali- Sam Ratulangi University.

Fatimawali

Pharmacy Study Program, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado, Indonesia.

A cross-sectional study was conducted between December, 2013, and May, 2014, to determine the prevalence and antibiotic resistance feature of Salmonella isolated from broilers slaughtered in Debre Zeit and Modjo towns, Ethiopia. A total of 384 caecal content samples were collected for microbiological examination following the standard techniques and procedures outlined by the International Organization for Standardization to isolate Salmonella. The sensitivity of the isolates subjected to nine antimicrobials was tested by the Kirby–Bauer disk diffusion method. The overall prevalence of Salmonella was 14.6%, and its occurrence differ significantly by farm (). The occurrence of the bacteria was not statistically different in the midland (15.2%) and lowland (13.3%) () and between males (13.5%) and females (15.6) (). Of the 50 isolates, 48 were resistant to at least one drug. Multidrug resistance was recorded in 43 (86.0%) of the isolates. The study demonstrated considerable prevalence and high antimicrobial resistant Salmonella in exotic chicken and indicates the potential importance of chickens as source of foodborne salmonellosis and multiple antimicrobial resistance of Salmonella. Improving the hygienic practice of farms could help to reduce the occurrence of Salmonella in farms. Further studies are needed to describe the risk factors associated with the emergence of drug-resistant Salmonella in chicken.

1. Introduction

Mercury is known as one of the heavy metals that is very toxic in the environment and can affect human and animal health. It exists in nature in three different forms with different toxicity, usage, and properties. The three forms of compounds are organic mercury, inorganic mercury, and elemental or metallic mercury. Despite its toxic properties, mercury is still widely used by the community in North Sulawesi as an ingredient to extract gold from the soil or ore as amalgam. Unfortunately, the waste from this activity is discharged freely without processing beforehand to be more friendly to the environment. children showed symptoms of ataxia. Other adverse effects that can arise due to mercury exposure include neurotoxicity, nephrotoxicity, teratogenicity (Minamata disease), increased risk of a heart attack and hypertension, cancer, and gene mutation. Mercury detoxification is one way to reduce mercury pollution, for example, by using mercury-resistant bacteria. Previous studies.

2. Materials and Methods

2.1. Study Area and Sample Collection

Soil samples were collected from traditional mining sites which used mercury metal to extract gold from ore in North Tanoyan Village, Bolaang Mongondow Regency, North Sulawesi, at an altitude of 2000 feet (500 meters) above sea level. At that location, there are three traditional gold minings which have been operating for more than 10 years. The first sample was taken from the mining waste disposal hole (location A), and the second sample was taken from the rice field (location B) which was about 100 meters from the mine waste disposal site. Sterile polyethylene tubes were used as soil containers. Samples were taken to the laboratory using a cooling box for further analysis of mercury content and bacterial

Note: This work is partly presented at “Clinical Microbiology 2018” on July 16-17, 2018 at Melbourne, Australia

composition, community structure, and diversity.

2.2. Measurement of Mercury Levels of the Samples

For mercury content analysis, 0.20 g of each soil was extracted with 10 mL mixed solution (2 mol/L HNO3 and 4 mol/L HCl) in a Teflon tube at 95°C for 2 h. The total amount of Hg in these extracts was determined via cold vapor atomic fluorescence spectrometry (CVAFS) (USEPA-3050-B and USEPA 245.7).

2.3. DNA Extraction, PCR Amplification, 454 Pyrosequen

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2.3. DNA Extraction, PCR Amplification, 454 Pyrosequen cing, and High-Throughput Sequencing Data Processing

The genomic DNAs (gDNAs) of bacteria were extracted from soil using ZymoBiomics DNA Mini kit (Zymo Research) according to the protocol provided by the manufacturer. Amplification of hypervariable V3-V4 regions of 16S rRNA were performed using MyTaq™ HS Red Mix (Bioline, BIO-25044) in Agilent SureCycler 8800 Thermal Cycler. The reaction conditions were as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 15 sec, annealing at 52°C for 30 sec, extension at 72°C for 45 sec, and then followed by final extension at 72°C for 3 min. Preparation of 16S rRNA libraries and bio informatics analysis were performed following the previous research.

2.4. Analysis of Bacterial Diversity

The alpha and global beta diversities of the bacterial gut were calculated and analysed using PAST3 v. 3.24.

3. Results and Discussion

3.1. Mercury Level of Samples

Mercury concentrations in both soil samples were analysed using CVAFS. The soil sample in location A had a high mercury concentration of 230 mg/kg (230 ppm). The soil sample obtained from location B which located 100 meters from location A had a much lower mercury concentration of 3.98 mg/kg (3.98 ppm). Both locations were separated by highways, but connected by a river. Vishnivetskaya et al. reported that with increasing distance from high levels of mercury-contaminated locations, inorganic mercury levels decreased, while Me-Hg levels increased, indicating mercury is a bioavailable compound and can be accessed by resident microorganisms. Revis et al. suggested that an acceptable limit of soil mercury was 72 ppm. The World Health Organization (WHO) suggested that the provisional tolerable weekly intake (PTWI) of mercury is 1 µg/kg body weight. The mercury in paddy fields may contribute to the level of mercury in rice which needed to be studied further. Feng et al. reported that the main exposure of Me-Hg in human was through the frequent consumption of rice meals. Long-term con-

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sumption of mercury-contaminated rice grain may further pose serious health risks.

3.2. Bacterial Composition

Metabarcoding analysis of 16S rRNA V3-V4 regions revealed that there were 57,031 reads (2,694 OTUs) in the sample from location A and 33,080 reads (2,759 OTUs) in the sample from location B, both consisting of 15 phyla of the kingdom bacteria and 2 phyla of the kingdom archaea (Crenarchaeota and Euryarchaeota) with a very limited amount in both locations. Phyla abundances in both locations are presented in Figures 1 and 2. Firmicutes (50%) was the most abundant phylum at location A, followed by Proteobacteria (24%). may be caused by different physical and chemical factors and soil nutrients in each sample that affected bacterial growth.

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