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IMPACT OF METAL CONTAMINATION TO THE EMERGENCE OF (MULTI) ANTIBIOTIC RESISTANT BACTERIA IN THAI NGUYEN, VIETNAM

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SUMMARY

Heavy metal contamination in the environment due to anthropogenic activities in industrial and agricultural areas represents stress for the living ecosystem including soil microorganisms. The adaptive response of bacterial communities to these pollutants can be observed through the increasing proportion of resistant bacteria. Our study aimed at evaluating the effect of metal contamination to the emergence of bacteria resistant to 3 common antibiotics, ie., gentamycin, sulfamethoxazole, and norfloxacin in Tan Long Pb-Zn mining site, Thai Nguyen province by a culture-dependent approach. The results showed that under moderated Pb contamination, a significant increase in prevalence of gentamycin-resistant bacteria was observed. Antibiotic susceptibility test showed 45% of isolates resistant to at least two antibiotics among gentamycin, norfloxacin, tetracyclin, imipenem, andsulfamethoxazole, and 10.4 % of isolates resistant tho three antibiotics. More detailed investigations in molecular analysis are needed to identify and genetically characterize of multi-drug resistant isolates as well as to better understand the link between metal resistance and (multi)resistance to antibiotics.

Keywords: *antibiotics, antibioticresistant, soil, metal contamination, Pb*

INTRODUCTION

In the last decades, the ecological threats caused by the contamination of ecosystems by metals become very preoccupying, as a result of anthropogenic activities such as mining and metallurgic industry. The adaptive response of bacterial communities to these pollutants can be observed through increasing proportion of resistant bacteria. Multi-resistance to antibiotics [i.e. "Multiantibiotic resistance" (MAR) or in particular, “Multidrug resistance” (MDR)] is a growing preoccupation and currently constitutes one of the major threats to public health worlwide. The hospital environment has long been known to be as a central place for the development and spread of antibiotic resistance. Nevertheless, outside the hospital environment, the literature also indicated that antibiotic-resistant bacteria are present in increased proportion in highly anthropogenic environments, including different chemical stresses due to the presence of antibiotics, organic compounds (biocides, hydrocarbons) and metals in the environment may be the cause of this selection (Alonso *et al.* 2000; Allen *et al.*, 2010; Berg *et al.*, 2010; Deredjian *et al.*, 2011; 2016). Ragarding the metal contamination of soil, there is growing concern that it functions as a selective agent in the proliferation of antibiotic resistance by the co-selection of resistance mechanisms (Seiler, Berendonk, 2012). Exposure to metals could select not only bacteria that are resistant to these pollutants, but also to antibiotics. The resistance to antibiotics and metals can be co-selected by different processes: i) co-resistance due to the presence of different resistance determinants on the same genetic element (for example, plasmid, transposon, integron, genomic islands etc…; ii) cross-resistance due to the presence of the same genetic determinant responsible for resistance to antibiotics and metals; iii) co-regulation due to the acting of metal or antibiotic as an inducer of a common regulator system responsible of various metal and antibiotic-resistant determinant expression (Ducret *et al*.,2016). The two last mechanisms, cross-resistance and co-regulation, specifically involve efflux pumps (Alvarez-Ortega *et al.*, 2013). These efflux systems are important in prokaryotes and can catalyze the active efflux of many compounds including antibiotics, organochlorine compounds or metals (Alvarez-Ortega *et al.*, 2013). These efflux pumps largely contribute to the acquisition of the MAR phenotype. They have the characteristics of being unspecific with respect to their substrates (Maseda *et al.*, 2011). These proteins can represent 6 to 18% of the membrane transporters found in bacteria and are grouped into five main families: Multidrug And Toxic Compound Extrusion (MATE), ABC (ATP Binding Cassette), MFS (Major Facilitator Superfamily), SMR (Staphylococcal MultiResistance) and RND (Resistance Nodulation Division) (Alvarez-Ortega *et al.*, 2013; Youenou *et al.*, 2015). A study by Berg *et al.* (2010) has shown that the high levels of Cu pollution co-selected bacteria resistant to Cu and different antibiotics. Compared to susceptible isolates, Cu-resistant isolates exhibited significantly higher resistance to 5 of the 7 tested antibiotics (tetracycline, olaquindox, nalidixic acid, chloramphenicol, and ampicillin) (Berg *et al.*, 2010). Moreover, Seiler, Berendonk (2012) showed that Hg, Cd, Cu, and Zn were most involved in the co-selection mechanisms of antibiotic resistance in soils or aquatic systems. According to these authors, the emission of these metals into the environment at certain thresholds (i.e "minimum co-selective concentration") could trigger a co-selection of antibiotic resistance.

Vietnam is a developing country in Southeast Asia with abundant and diversified natural mineral resources. However, intensive mining in recent years has resulted in depletion of resources and environmental degradation, of which pollution by metals is an alarming problem (Dang, 2010). A recent study showed that out of the 5,000 mining sites reported, 90 remain active resulting in abandoned mine areas of 3,749 ha. For example, the total area used for Sn exploitation is 300 ha, but only 20% of these have been restored (Anh *et al.*, 2011). Among the northern provinces of Vietnam, Thai Nguyen is considered as one of the capitals for mining industry with 177 operating sites currently active, including limestone, clay, coal, iron ore, dolomite, as well as heavy metal mines such as Ti, W, Pb, Sn. and Au. Although metal mining brings economic benefits, the environmental impact including contamination of soil and ground water together with the loss of biodiversity is inevitable with the use of obsolete technology and the lack of preliminary treatment (Dang *et al.*, 2010). In Ha Thuong, Dai Tu (Thai Nguyen Province), the measured concentration of As in soils was found in between 3102 and 6754 mg/kg, the highest contamination level of As in Vietnam (Anh *et al.*, 2011), and the Cu level in soil reached 3150 mg/kg (Pham *et al.*, 2017). High levels of Pb, Cd, and Zn was found in Dong Hy, an active mining site in Tan Long, at 4337, 419 and 17565 mg/kg, respectively (Dang *et al.*, 2010). Therefore, it is very important to evaluate whether the metal contamination in these mining sites applies as a selection pressure on bacterial populations leading to the emergence of MAR bacteria. This study aimed at determining the impact of heavy metal pollution to the emergence of MAR bacteria in Thai Nguyen province, Vietnam by a culture-dependent approach. Antibiotic susceptibility profile of some (multi)resistant bacterial isolates was also investigated.

MATERIALS AND METHODS

Site studied and sampling information

From Thai Nguyen Province, four types of sites were collected: 1) mining area (Tan Long, Dong Hy district); 2) mining area with farms (Tan Long, Dong Hy district); 3) farms (Thai Nguyen city); 4) areas without mining and farms (Thai Nguyen city - considered as conltrol samples). From each site, 15 soil samples from the upper layer (0–15 cm) at different locations were collected and stored in ambiant temperature for no longer than one week before analysis. Soil physico-chemical properties were analysed by Soil and Fertilizer Research Institute (Hanoi, Vietnam). Detailed sampling information were shown in Table 1.

Table 1. Summarry of soil sampling information and soil physico-chemical properties.

Properties	**Site**	**Tan Long (Pb-Zn mine)**	**Tan Long (chicken farms)**	**Thai Nguyen city (control)**	**Thai Nguyen city (chicken farms)**
	Location	21°36’00”N, 105°50’00”E	21°35’55”N, 105°48’45”E	21°43’49”N, 105°51’16”E	21°43’37”N, 105°51’56”E
Properties	Code Unit	TL-M	TL-G	TN-C	TN-G
Clay (< 2 μm)	g/kg	276.06	197.16	133.5	166.7
Silt	g/kg	461.82	377.89	469.92	388.5
Sand	g/kg	122.12	484.95	286.58	313.4
Organic matter	g/kg	45.924	23.2065	16.999	22.01
Organic Carbon (C)	g/kg	26.574	21.8497	19.8345	16.02
Total Nitrogen	g/kg	2.0511	0.20087	0.9434	0.75
C/N	-	11.18	9.5347	9.256	10.88
pH	-	4.5838	4.4414	7.2713	6.87
(CEC*) cobaltihexamine	cmol+/kg	11.352	8.0242	9.8345	7.017

*CEC: Cation exchange capacity

Evaluation of metal level in soil

In order to evaluate the metal pollution level of soil samples, the common metals (Pb, Zn, Cd, Cu, As, Fe) content in each sample was measured by ICP-MS methods (Voica *et al.*, 2012; Manh *et al.*, 2018). Briefly, each 0.5 g of soil sample was digested by a mixture of 3 mL concentrated H_2SO_4 95%, 10 drops of $HClO_4$ and 10 mL concentrated HNO_3. The samples were then heated (from 150–200°C) until the solution was transparent. The digested solution was let cool to room temperature, filtered with quantitative paper (Whatman, 11µm) to remove the sand. The residues were then filled up to 25 mL with mili-Q water with 2% HNO_3 and skept in fridge until analysis. The analysis was performed by ICP-MS (Perkin Elmer ELAN 9000, Perkin Elmer Sciex Penlivia Canada). The ICP-MS conditions were RF power: 1300W; plasma gas flow: 16L/min; Auxiliary gas flow: 1.25L/min; nebulizer gas flow: 0.9L/min; nebulizer: cross-flow; spray chamber: PFA double gas; monitored ion M/z (Pb: 208, Zn:66, Cu:63, Cd:111, As:75, and Fe:57). The quantification was measured by external calibration curve which was prepared from a high purity ICP-multielement calibration standard (Perkin Elmer Life and Analytical Sciences). The data were treated and illustrated in mg/kg of soil.

Determination of antibiotic level in soil

After the collection, all samples were broungt back to the laboratory and stored at 4°C until analysis. In sample treatment step, 10 g of each homogenized sample was weighed into a 50 mL QuEChERS centrifuge tube which contained 4 g $MgSO_4$, 1 g NaCl, 1 g Na_3Citrate and 0.5 g Na_2Citrate. After that, 10 mL of Acetonitrile (ACN) was added into the QuEChERS tube which then submitted to ultrasound at room temperature, 40Khz for 10 minutes by VWR Ultrasonic system (Avantor, USA). The mixture then was centrifuged at 5000 rpm for 10 minutes. Finally, the supernatant was filtered through 0.45 µm and dried under nitrogen stream at 40 °C until droplets were formed and refill with acetonitrile up to 1 mL. The concentration of four antibiotics (Imipenem, Ciprofloxacin, Trimethoprime, and Sulfamethoxazole) in soil were analyzed by LC-MS/MS system (WATERS, USA). Analyte separation was carried-out on a C18 reverse phase column (100 mm x 2.1 mm; 1.7 µm) (WATERS, USA). The following gradient was run using 0.5% aqueous formic acid (FA) (solvent A) and acetonitrile with 0.5% FA (solvent B): 0 min, 0% B; 1 min, 0% B; 3 min, 70% B; 4 min, 70% B; 4.1 min, 0% B; 6 min, 0% B. The flow rate was 0.3 ml/min, respectively and 10.0 µL of sample extracts were injected.

In the TQD mass spectrometer, nitrogen was used as the cone gas (65 L/h) and as the desolvation gas (645 L/h). The desolvation temperature and source temperature were 300ºC and 200ºC respectively. The collision gas used was Argon at pressure of 3.2×10^{-3} mBar. The ionization was used as mode positive electrospray ionization (ESI+) and the spectrometer was performed in the multiple reaction monitoring (MRM) mod with the dwell time of 25 ms. The ESI-MS/MS parameters were optimized by direct injection of individual standard solution which concentration was around 1 mg/L using a syringe pump.

Enumeration of resistant cultured heterotrophic bacteria

The total heterotrophic microflora was counted on the medium TSA 1/10 (Tryptic Soil Agar diluted ten times) (Sigma-Aldrich, USA), supplemented with antifungal agent (final concentration of cycloheximide 200 mg/L). Heterotrophic resistant bacteria were enumerated on this same medium supplemented with antibiotics. The following antibiotics were tested because of their utilization in farming activities: gentamicin (aminoglycoside family) at the final concentration of 32mg/L, norfloxacin (family of fluoroquinolones) at the final concentration of 10 mg/L and sulfamethoxazole (families of sulfonamides) at the final concentration of 60 mg/L. The concentrations of antibiotics or metals for the isolates were chosen on the basis of literature data as well as on the data of the French Society of Microbiology concerning the distinction between sensitive phenotype and resistance for different pathogens.

The bacteria were retrieved from the soil by grinding 5 g of sample in 50 mL of saline solution (0.8% NaCl) for 1 min in a Waring Blender appareil (Eberbach Coorporation, New Hampshire, USA). The homogenized soil suspension was serially diluted in 0.8% NaCl and 100 µL of the 10^{-1} and 10^{-2} dilutions were plated on the appropriate antibiotic-containing dishes for the resistant heterotrophic microflora and the 10^{-3} and 10^{-4} were plotted for total heterotrophic microflora. Experiments were performed in triplicate and dishes with 30 to 100 bacterial colonies were counted after 2 to 5 days of incubation at 28 °C.

Antimicrobial susceptibility evaluation

The resistant isolates representing different morphotypes were selected. Antimicrobial susceptibility testing was performed using the disc diffusion protocol according to the Clinical Laboratory Standards Institute (CLSI, 2015) guidelines. Tests were done on Mueller Hinton agar (Biokar, India), to commonly used antibiotics: gentamycin (GEN), norfloxacin (NOR), sulfamethoxazole (SMX), imipenem (IMP) and tetracycline (TET). Antimicrobial activity of each antibiotic was tested for all isolates and results will be interpreted according to the guidelines provided for antimicrobial disc susceptibility tests by CLSI and results were recorded as Susceptible (S), Intermediate Resistant (I) and Resistant (R). The isolates resistant to at least 3 tested antibiotics will be considered as MAR.

Statistical analysis

The prevalence of resistant bacteria and the proportion of MAR isolates in different samples were analyzed using ANOVA followed by Tukey's Honest Significant Difference (HSD) post hoc tests to determine the significant discrimination between two groups contaminated and non contaminated soils. Significant differences were achieved for those analyses conducted at $p < 0.05$.

RESULTS AND DISCUSSION

Metals and antibiotic level in soil

The result in Table 2 showed that the two Tan Long soils are moderately contaminated in Pb with higher concentration than the limit to be allowable for industrial soil in Vietnam (391.2 mg/kg^{-1} and 324 mg/kg^{-1} for TL-M and TL-G, respectively), while the two sites in Thai Nguyen city could be considered as non-polluted.

Concentration of four antibiotics (CARBA, CIP, TRIM, SMX) in soils samples were evaluated. However, we couldn't detect any antibiotic even at minor concentration from these soils. The soil collections were realised three times in different sites, however the same observation was found. The results showed that these examined soils from Thai Nguyen, even at farming sites, were not contaminated with the four tested antibiotics (CARBA, CIP, TRIM, SMX).

Prevalence of antibiotic resistance in total heterotrophic microflora

The abundance of heterotrophic bacteria resistant to each of the antibiotics tested norfloxacin (NOR), gentamycin (GEN), and sulfomethoxazole (SMX) from soils collected at mining site Tan Long, and from the control site (Thai Nguyen city) with or without chicken farms were evaluated (Table 3). Interestingly, resistant bacteria were found in all soil samples with variation in abundance between mining and control site. The total heterotrophic microflora count varied from $(165.22 \pm 69.09) \times 10^5$ (CFU/g) for TN-G to $(251.94 \pm 138.87) \times 10^5$ for TN-G. The highest values of resistant bacteria to SMX were found at $(14.54 \pm 9.27) \times 10^5$ (CFU/g) in the mining site (TL-M) whereas for the chicken farm in the same region (TL-G), this value was found in lowest level. Regarding two other antibiotics, GEN resistant bacteria was also counted in highest concentration $(5.57 \pm 1.79) \times 10^5$ (CFU/g) and $(5.33 \pm 1.61) \times 10^5$ (CFU/g) in the two mining site (TL-M, TL-G)), while NOR resistant was more increased in the non-polluted farming site (TN-G) (Table 3). However, no signigicant difference between the Tan Long mining site (TL-G, TL-M) and the control site (TN-C, TN-G) nor between the samples with or without chicken farm was found (ANOVA followed by Tukey HSD, $p > 0.05$).

Table 2. Soils heavy metal level (TL-M: Tan Long mining site; TL-G: Tan Long chicken farm; TN-M: Thai Nguyen city; TN-G: Thai Nguyen chicken farm).

Samples	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)	Cd (mg/kg)	As (mg/kg)
TL-M	144.18	134.25	391.2	0.524	14.9
TL-G	127.27	89.56	324	0.655	13.4
TN-C	102.35	113.75	137.28	0.786	2.6
TN-G	151.3	125.25	92.56	0.524	20.6
Limit for industrial soil *	300	300	300	10	25

* According to Vietnam National technical regulation on the allowable limits of heavy metals in the soils (QCVN 03-MT 2015/BTNMT)

Table 3. Antibiotic resistant bacteria count and total heterotrophic microflora count of four samples (TL-M: Tan Long mining site; TL-G: Tan Long chicken farm; TN-M: Thai Nguyen city; TN-G: Thai Nguyen chicken farm; NOR: Norfloxacin; GEN: Gentamycin; SMX: Sulfamethoxazole).

Sample	NOR resistant (x 10^5 CFU/g) (n = 5)	GEN resistant (x 10^5 CFU/g) (n = 5)	SMX resistant (x 10^5 CFU/g) (n = 5)	Total heterotrophic microflora (x 10^5 CFU/g) (n = 5)
TL-M	8.26 ± 1.99	5.57 ± 1.79	14.54 ± 9.27	177.23 ± 45.12
TL-G	13.31 ± 10.09	5.33 ± 1.61	10.40 ± 7.35	185.22 ± 69.09
TN-G	15.25 ± 8.09	5.09 ± 0.60	11.86 ± 7.73	251.94 ± 138.87
TN-C	10.93 ± 4.27	1.53 ± 0.97	11.76 ± 5.93	198.40 ± 51.62

Concerning the proportion of resistant bacteria, the same tendance was observed. For SMX, the highest prevalence of resistant phenotypes was observed in the Tan Long mining site (TL-M, 8.20 % ± 5.23) whereas the most abundant in NOR resistance was accounted for the chicken farm soil in Tan Long (TL-G, 7.18% ± 2.44), however there was no significant difference in proportion of resistant bacteria in both SMX and NOR between the mining site (TL-M and TL-G) and the controls (TN-C and TN-G) as well as between the chicken farm soil and the soil without farming activities (ANOVA test followed by Tukey HSD, $p > 0.05$). This observation does not allow us to conclude the selection of bacterial communities resistant to NOR and SMX under Pb pollution condition. On the other hand, the GEN resistant bacteria were found at significant higher proportion in the two mining soils (3.14 % ± 1.01, TL-M and 2.88 % ± 0.87, TL-G) than in the two non-polluted soils (TN-G and TN-C) (ANOVA test followed by Tukey HSD, $p < 0.05$). Meanwhile, there was no significant difference between the chicken farm soil and the soils without farming activities. According to our knowledge, the influence of Pb pollution on the emergence of GEN resistance was not reported yet. The fact that GEN resistant bacteria was detected both in higher concentration and in increased prevalence in the two Pb-contaminated mining samples whatever presence of chicken farm or not suggested that Pb at moderated concentration would be the selective pressure for the GEN resistant bacteria. This result is in accordance with the hypothesis in the literature that metal pollution would induce the selection of some antibiotic-resistant bacteria (Berg *et al*., 2010; Pham *et al*., 2018). Previous study found that emerging resistant microbiota could relate to the acidic characteristic soils and the bioavailability of the metal including Pb (Caliz *et al*., 2013).

Curiously, the two mining soils (TL-G and TL-M) possess an acidic property (pH 4), however more investigation about the bioavailability of Pb in these samples should be performed to allow us to go further in the interpretation of these results.

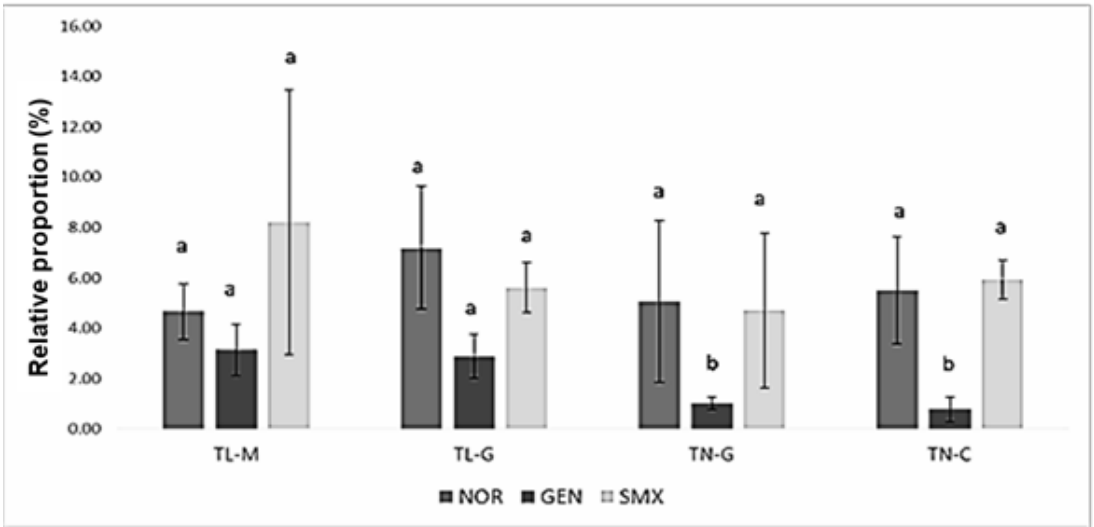

Figure 1. Prevalence of antibiotics resistant bacteria in different soils in Thai Nguyen, Vietnam. The average values of five replicates were used to represent the distribution within each soil. Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$). Error bars represent standard deviation (n=5). (TL-M: Tan Long mining site; TL-G: Tan Long chicken farm; TN-M: Thai Nguyen city; TN-G: Thai Nguyen chicken farm; NOR: Norfloxacin; GEN: Gentamycin; SMX: Sulfamethoxazole).

Multiantibiotic resistant of the selected isolates

Twenty-nine resistant isolates representing different morphotypes were selected. Antimicrobial susceptibility was tested for the all isolates using five common antibiotics Norfloxcin, Tetracycline (TET), SMX, GEN and Imipenem (IPM) and results were interpreted according to the guidelines provided for antimicrobial disc susceptibility tests by Clinical and Laboratory Standards Institute. Results were recorded as Susceptible (S), Intermediate Resistant (IR) and Resistant (R) (Table 4). The isolates resistant to at least 3 tested antibiotics were considered as MAR.

Among the 29 selected isolates, 3 (10.4 %) were identified as resistant to at least 3 tested antibiotics (Fig. 2), thus considered as MAR. Curiously, these 3 isolates were all detected in mining soils. Approximately 14.3 % (1 out of 7) of resistant isolates in farm soil from Tan Long mining site were MAR while for soil without farm, this proportion reached 22.2 % (2 out of 9 isolates). Forty-five percent of isolates were resistant to at least 2 antibiotics: five isolates are resistant to NOR and SMX, 4 isolates were resistant to SMX and GEN, or to GEN and NOR, and 1 isolate were resistant to SMX and TET. This observation was in accordance with the above result, indicating the high proportion of resistant bacteria to these 3 antibiotics in mining soil samples. Imipenem resistance was found in all 4 soil samples whereas TET resistance was only detected in 3 samples. Morever, no MAR isolates were detected in the control site, even at chicken farm, suggesting that heavy metal pollution (mainly Pb) would favor the emergence of MAR bacteria. However, the lacking of antibiotic data in soil didn't allow us to evaluate whether the synergic effect of metal and antibiotic contamination led to the emergence of MAR bacteria or not.

Table 4. Results of antibiotic disc diffusion tests for the selected resistant isolates from different soil samples of Thai Nguyen, Vietnam. (TL-M: Tan Long mining site; TL-G: Tan Long chicken farm; TN-M: Thai Nguyen city; TN-G: Thai Nguyen chicken farm; NOR: Norfloxacin; GEN: Gentamycin; SMX: Sulfamethoxazole; TET: Tetracyclin; IPM: Imipenem).

Soil samples	Resistant Isolates	Antibiotic susceptibility				
		NOR	GEN	SMX	TET	IPM
TL-M	TL-M1	S	R	S	S	R
	TL-M2	S	S	S	S	S
	TL-M3	R	R	S	S	S
	TL-M4	**R**	**R**	**R**	**S**	**S**
	TL-M5	R	S	S	S	R
	TL-M6	I	S	R	S	S
	TL-M7	**R**	**R**	**R**	**S**	**S**
	TL-M8	R	I	I	S	S
	TL-M9	S	R	R	S	S
TL-G	**TL-G1**	**R**	**S**	**R**	**S**	**R**
	TL-G2	S	S	I	S	S
	TL-G3	R	I	R	S	S
	TL-G4	R	S	I	S	S
	TL-G5	S	R	R	S	S
	TL-G6	I	I	S	I	S
	TL-G7	S	S	R	R	S
TN-C	TN-C1	S	S	S	S	R
	TN-C2	R	S	S	S	S
	TN-C3	S	S	S	I	S
	TN-C4	I	R	I	S	S
	TN-C5	R	S	S	S	S
	TN-C6	S	S	S	R	S
	TN-C7	S	S	S	S	S
TN-G	TN-G1	R	R	S	I	S
	TN-G2	S	S	R	S	S
	TN-G3	I	R	S	S	S
	TN-G4	S	S	R	S	S
	TN-G5	R	S	S	S	R
	TN-G6	S	S	S	S	S
	TN-G7	R	R	S	S	S

Figure 2 showed the antibiogramme of the three MAR strains on Mueller Hinton Agar. It's worth noting that althought representing the same MAR profile (resistant to NOR, SMX and GEN), TL-M4 and TL-M7 strains were morphologically different. Colony of strain TL-M4 appeared yellow in color and fairly circular in shape, its Gam staining showed a rod shape Gram-negative bacteria (Figure 3b). The TL-G1 strain were rod shape Gram-positive bacteria, its colony had a dull color and a translucent, smooth surface, whereas the TL-M7 strain was Gram-positive cocci with dry and opaque colony. (Figure 3a, 3c). This suggests that these MAR strains belong to different bacterial group. Further analysis would be needed in order to identify and genetically characterize of these strains.

Antibiotic resistances are widespread in the contaminated environment in response to the presence of antibiotics due to different anthropogenic activities (Martinez, 2009). Several researches have reported that antibiotic resistance capacities could be elevated in heavy metal contaminated environments (Stepanauskas *et al.*, 2005, Wright *et al.*, 2006, Deredjian *et al*, 2011). In Vietnam, study on the influence of metal contamination to the emergence of (multi)drug resistant bacteria is scarse. Here we studied the prevalence of antibiotic resistant from Tan Long mining site, and evaluated the antibiotic suscepbility of some resistant isolates. So far, our report is among the first ones evaluating the impact of Pb contamination on antibiotic resistant bacterial population in Thai Nguyen province. Recent study by Pham *et al.* (2017) indicated an enrichment in genera known as opportunistic human pathogens and antibiotic resistant, including *Ralstonia*, *Acinetobacter*, *Burkholderia* and *Mycobacterium* in *P. vittata* rhizosphere at Ha Thuong mining site under moderate Pb and Zn contamination. In another geological context, the selection of *Stenotrophomonas* and *Pseudomonas* in French agricultural soils contaminated with Zn and Pb was observed (Pham *et al.*, 2018). Deredjian *et al.* (2010) also detected the high prevalence of *Stenotrophomonas* and *Pseudomonas* among the MAR isolated from various French agricultural soils contaminated with Pb and Zn. Although we could not acquire the data about antibiotic concentration and the bioavailability of Pb in soil, the fact that the prevalence of GEN resistant bacteria was significantly elevated in Pb-contaminated soils suggested the selection of GEN resistant bacteria under Pb pressure. Further molecular approach in GEN resistant isolates such as TL-M4, TL-M7 would be envisaged to understand how metal pollution favors the (multi)resistance to antibiotic.

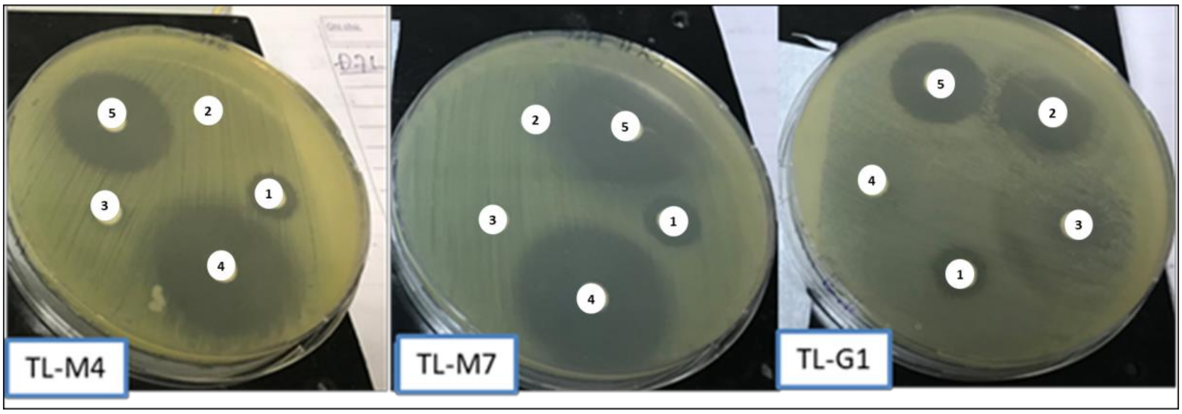

Figure 2. Antimicrobial susceptibility test of the three MAR strains from Tan Long mining site with chicken farm (TL-G1) and without farm (TL-M4 and TL-M7) (1: Norfloxacin; 2: Gentamycin; 3: Sulfamethoxazole;; 4: Imipenem; 5: Tetracyclin).

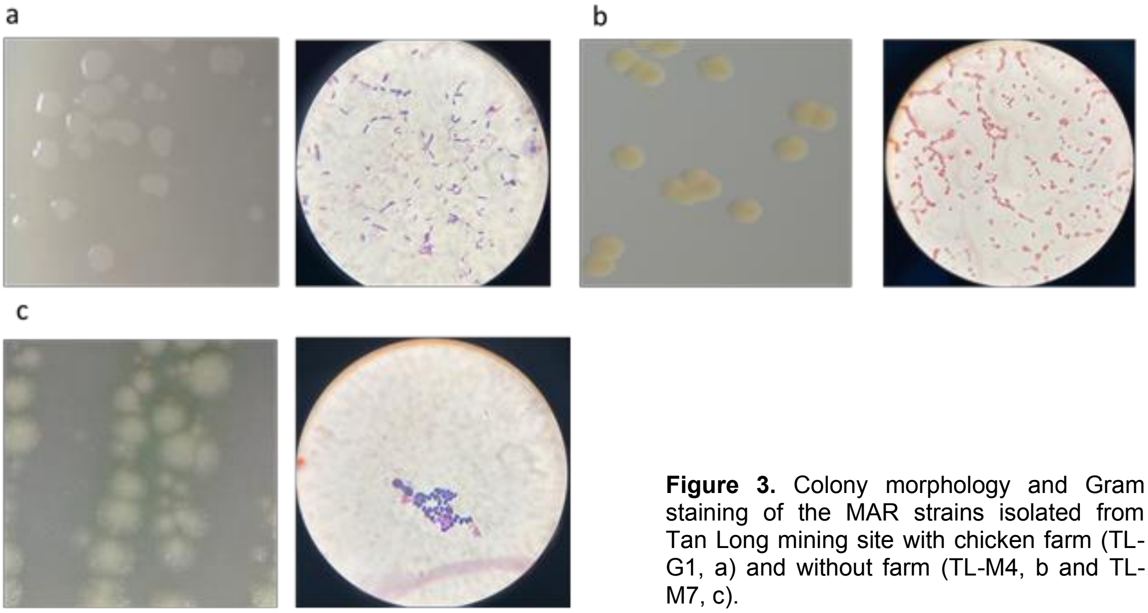

Figure 3. Colony morphology and Gram staining of the MAR strains isolated from Tan Long mining site with chicken farm (TL-G1, a) and without farm (TL-M4, b and TL-M7, c).

CONCLUSION AND PERSPECTIVE

The present study showed a significant increase of prevalence of GEN resistant bacteria in Pb-conaminated soil from Tan Long mining site, Thai Nguyen province. Antibiotic susceptibility test showed 45% of isolates resistant to at least two antibiotics including GEN, NOR, TET, IPM, and SMX and 10.4 % of isolates resistant to three antibiotics. More detailed investigation in molecular biology are needed to identify and genetically characterize of MAR isolates, as well as to better understand the link between metal resistance and (multi)resistance to antibiotics.

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