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The Presence of Metals and Antibiotics Resistant Bacteria in Arable Manure Soils

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Heavy metals and antibiotics resistance are intense public interest owing to their pollution in the environment and potential deleterious effects on human health. A total of 15 isolates of *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli* (50 manure garden soils) were tested for their resistance against certain heavy metals and antibiotics. Minimum inhibitory concentrations of Pb^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} for each isolate were also determined. *B. subtilis, B. cereus, P. aeruginosa* and *E. coli* exhibited high tolerance to minimum inhibitory concentrations of 3,200 μg/ml for Pb^{2+}, 400 μg/ml for Cu^{2+} and Co^{2+}, and 100 μg/ml for Zn^{2+} in agar plates. All the isolates were highly resistant to lead and they showed 100% growth in 3,200 μg/ml concentration. It was observed that all isolates were resistant to a wide range of antibiotics namely clindamycin, ceftazidime, aztreonam and vancomycin.

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PACS/topics: heavy metals, antibiotics, resistance, manure soils

1. Introduction

Various animal manure is applied in agricultural areas to increase soils fertility. However, this soils fertility causes also serious environmental problems, such as antibiotics (tetracycline, oxytetracycline, and chloramphenicol) and metal residues (Co, Hg, Cd, Pb, Cu, Zn, As, U, and Cr) to arable manure soils [1–3]. These residues are found in arable manure soils. Also, antibiotics and heavy metals pollution in arable soils and ecosystem have become risk to natural life and public health [4–6]. Recently, higher levels of radioactive, antibiotic, and heavy metal residues were detected in organic vegetable fields and intake of these residues by food products affects food quality and safety [7–10].

These residues that pollute the natural environment and agricultural soils may contain antibiotic resistance genes and selection of resistant bacterial populations in soils. Resistance genes for antibiotics and heavy metals are transported by the moving genetic elements such as plasmids, integrons, and transposable elements [11]. Antibiotics and heavy metals pollution in the agricultural area rapidly increase the number of antibiotic resistance genes (ARGs) in bacteria [12].

The objectives of this study were to investigate antibiotic and heavy metal resistance in isolates from Kırşehir manure garden soils. The results will be helpful in further revealing the ecological harm and human health hazard of complex pollutants.

2. Materials and methods

2.1. Sample collection

Manured soil samples were collected from surface to a depth of about 15 cm using sterilized polystyrene bags of 10 g from vegetables grown in manure garden soils different 10 areas of Kırşehir. Samples were transferred for microbial analysis.

2.2. Bacteriological isolation and identification

For isolation of bacteria from garden soils, 10 g of soil samples was suspended in 90 ml of sterile normal saline solution and shaken vigorously for 10 min. Dilution were plated on NA medium agar. This study was made to evaluation the VITEK 2 system (bioMérieux) for identification.

2.3. Antibiotic sensitivity test

All isolates were tested for its sensitivity to antibiotics by means of a disc diffusion method. The bacteria were investigated using antibiotics disc containing clindamycin, ceftazidime, aztreonam, vancomycin, gentamicin, imipenem, chloramphenicol, amikacin, and cefuroxime were placed on the surface of the agar plates and incubated at 37 °C for 24 h [13].

2.4. Determination of the MIC of heavy metals

The minimal inhibitory concentration (MIC) of metals such as lead (Pb), cobalt (Co), copper (Cu), and zinc (Zn) was determined for different bacterial isolates by the plate dilution method [13]. The growth was observed during 24–48 h at 37 °C. MIC of the metals for each isolate was determined by the plate dilution method [13].

3. Results and discussion

3.1. Antibiotic sensitivity

A total of 4 species of bacteria were isolated: the most common strains isolated from all samples were 7 *P. aeruginosa* (46.6%), 4 *E. coli* (26.6%), 2 *B. subtilis* (13.3%), and 2 *B. cereus* (13.3%). The resistance of 11 Gram-negative and 4 Gram positive bacterial isolates to 10 different antibiotics, and to four heavy metals, was investigated by agar diffusion. A high level of resistance against clindamycin, ceftazidime, aztreonam, and van-

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comycin (100%) was observed in manured garden soils. Furthermore, 86.6% of isolates showed resistance to cefuroxime, whereas 26.6% were resistant to both amikacin and ciprofloxacin. All bacterial isolates were susceptible to gentamicin, imipenem, and chloramphenicol (Table I). 100% of the *Pseudomonas* isolates were susceptible to chloramphenicol found in this study is in agreement with Shafiani and Malik [14]. Shigoli et al. [15] reported that they used enteric bacterial soil isolates most inhibited to chloramphenicol, cefotaxime, ciprofloxacin, and gentamicin. In this study, we observed clindamycin, ceftazidime, aztreonam, vancomycin and gentamicin were all highly active against both gram negative and gram positive bacteria. Ghaima et al. [16] reported vancomycin are effective against B. cereus isolated from soil samples contaminating diesel fuel.

TABLE I

Difference between the species in terms of antibiotic and heavy metal resistance.

Antibiotic [μg/disc]	*P. aeruginosa* ($n = 7$)	*E. coli* ($n = 4$)	*B. subtilis* ($n = 2$)	*B. cereus* ($n = 2$)	% resistance
clindamycin [2 μg/disc]	7(100)	4(100)	2(100)	2 (100)	15 (100)
ceftazidime [30 μg/disc]	7(100)	4(100)	2(100)	2 (100)	15(100)
aztreonam [30 μg/disc]	7(100)	4(100)	2(100)	2 (100)	15(100)
vancomycin [30 μg/disc]	7(100)	4(100)	2(100)	2 (100)	15(100)
gentamicin [10 μg/disc]	0	0	0	0	0 (0.00)
imipenem [10 μg/disc]	0	0	0	0	0 (0.00)
chloramphenicol [30 μg/disc]	0	0	0	0	0 (0.00)
amikacin [30 μg/disc]	4 (26.6)	0	0	0	4 (26.6)
ciprofloxacin [5 μg/disc]	4 (26.6)	0	0	0	4 (26.6)
cefuroxime [30 μg/disc]	5 (71.4)	4 (100)	2 (100)	2 (100)	13 (86.6)
Heavy metal [μg/ml]	*P. aeruginosa* ($n = 7$)	*E. coli* ($n = 4$)	*B. subtilis* ($n = 2$)	*B. cereus* ($n = 2$)	% resistance
Pb [200–3200 μg/ml]	7 (100)	3 (75)	2 (100)	2 (100)	14 (93.3)
Co [200–3200 μg/ml]	7 (100)	3 (75)	1 (50)	1 (50)	12 (80)
Cu [100–400 μg/ml]	7 (100)	3 (75)	1 (50)	1 (50)	12 (80)
Zn [100 μg/ml]	6 (85.7)	3 (75)	1 (50)	1 (50)	11(73.3)

3.2. Heavy metal resistance

MIC of Pb^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} for each isolate were also determined. A maximum MIC of 3,200 μg/ml for lead and 100 and 400 μg/ml for other metals were observed. The efficient isolates exhibited a high degree of tolerance to elevated concentrations of lead (3200 μg/ml). It was also tolerant to fairly high concentrations of copper and cobalt (400 μg/ml) and (100 μg/ml) for Zn^{2+} in agar plates (Table I). The study showed that Pb, Co, Cu resistance was present in 100% soil all *P. aeruginosa* isolates, whereas for *E. coli* the level was 75% of soil isolates. A heavy metal resistance pattern of Pb > Co and Cu = Zn was observed in *B. subtilis* and similarly for *B. cereus* Pb > Co and Cu = Zn. Previous study by Ghaima et al. [16] showed that the highest range was 400 mg/l for Pb and 350 mg/l for Zn soil contaminated with diesel fuel for *B. cereus*. In the same manner, a study by Khatun et al. [17] mentioned that *B. cereus* was most tolerable to Cd and Cr comparing to metals Co and Pb.

4. Conclusion

The present study revealed that heavy metals and antibiotics resistant bacteria were isolated from manure soil. These results notice that antibiotic resistant bacteria can be affected by the concentration of co-exposed heavy metals and will threaten natural life and human health through horizontal gene transfer. According to these results, antibiotic and heavy metal residues can also help to bring about human health and ecological damage.

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