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Presence of β-Lactamases Encoding Genes in Soil Samples from Different Origins

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Abstract The functional classification of β-lactamases is done through assessing their ability to hydrolyze specific β-lactams and its inactivation by inhibitors. This study investigated the β-lactamases encoding genes present in soil samples from different origins (landfill, preservation area, and soil from a farm). Genes codifying for ESBL enzymes bla_{SHV}, $bla_{TEM\text{-}116}$ and $bla_{OXA\text{-}1}$ were found in all analyzed samples. Gene for ESBL $bla_{CTX\text{-}M\text{-}14}$ was detected in the landfill and farm soil samples, but they were not found in the preservation area, while $bla_{OXA\text{-}48\text{-}like}$ was present just in the soil from the landfill. The gene for the MBL bla_{VIM} was found in the soil sample from a farm. The results indicate that bla_{SHV}, $bla_{TEM\text{-}116}$, and $bla_{OXA\text{-}1}$ genes are scattered in soils with and without potential contaminants; however, genes $bla_{CTX\text{-}M\text{-}14}$, $bla_{OXA\text{-}48}$, and bla_{VIM} were detected just in polluted areas.

Keywords β-lactamases · Soil · ESBL · Resistance

1 Introduction

The antimicrobial resistance is nowadays a concern due to the difficulty to treat human and animal infections (Levy 2002). Resistance is a mechanism acquired by microorganisms by changing their genetic structure, and the most common form of resistance to β-lactam antibiotics is the production of enzymes named beta-lactamases, which inactivate the antibiotics by cleavage of the β-lactam ring (Perez et al. 2007; Medeiros 1997).

According to Bush and Jacoby (2010), the β-lactamases functional classification is based on their ability to hydrolyze specific β-lactams and on the inactivation by inhibitors sulbactam, tazobactam, and clavulanic acid. Thus, they are classified into three main functional groups: group 1—cephalosporinases; group 2—serine β-lactamases; and group 3—metallo-β-lactamases (MBLs).

Group 1 is more active on cephalosporin than on benzylpenicillin and includes AmpC, CMY, ACT, DHA, FOX, and MIR enzymes. Group 2 is the largest and heterogeneous family, including all the extended-spectrum β-lactamases (ESBL) enzymes, whose main member are SHV, TEM, CTX-M, VEB, PER, GES, KPC, SME, and OXA. These enzymes hydrolyze penicillins and extended-spectrum cephalosporins, and some of them have activity on monobactams and carbapenems. Group 3 is structurally different from the other two groups due to their request for zinc at the active site and can hydrolyze all β-lactams, except the monobactams. The main enzymes of this last group are IMP, VIM, NDM, SPM, GIM, and SIM. These enzymes are distributed globally in nonfermentative bacteria and in the *Enterobacteriaceae* family (Bush and Jacoby 2010; Tsakris et al. 2009).

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Although the use of antibiotics is controlled in human and veterinary medicine and in agriculture, antimicrobial resistance genes were detected by Knapp et al. (2010) in different soil samples. Besides that, in 2011, Martínez and collaborators reported that in areas with greater human activity, the existence of bacteria from different sources leads to the propagation of resistance genes. According to D'Costa et al. (2006), this dispersion also can be occasioned due to the presence of antibiotic-producing organisms in soil.

In this way, the goal of this work was to verify which main β-lactamases encoding genes are present in soil samples and to determine if there is a major occurrence of these genes in soil samples from different origins.

2 Material and Methods

2.1 DNA Extraction from Soil Cultivable Bacteria

Three soil samples of distinct points from each area were mixed to obtain a single sample. One gram of each mixed soil sample was added in 5 mL of Luria-Bertani (LB) broth (Difco Laboratories, Detroit, MI, USA) at 37 °C for 24 h, and, after that, DNA extraction was done, using the QIAamp DNA Mini Kit (QIAGEN, Germany), according to the manufacturer's instructions. The concentration of the DNA was determined using a NanoDrop®1000 spectrophotometer (Thermo Scientific, USA).

2.2 PCR Screening for β-Lactamases and Sequencing of the Amplified Genes

PCR reactions were performed in the ProFlex™ PCR System (Applied Biosystems, Singapore) to investigate all β-lactamases listed in Table 1. Annealing temperatures, PCR primers, amplicon sizes, and references are also indicated in Table 1. A final volume of 50 μL was used and PCR conditions were performed as follows: initial denaturation at 94 °C (5 min), 35 cycles at 94 °C (1 min), annealing temperature at different temperatures (Table 1) for 1 min, extension at 72 °C (2 min), and an additional extension at 72 °C (7 min) using 200 ng of DNA and 1.25 U Taq DNA polymerase (Thermo Scientific, USA). Reactions without DNA were used as a negative control.

The strains *Escherichia coli* GES (bla_{GES}), *Klebsiella pneumoniae* ATCC® 700603 (bla_{SHV}), *E. coli* 8501 ($bla_{CTX\text{-}M\text{-}Gp1}$ and $bla_{CTX\text{-}M\text{-}Gp9}$), *K. pneumoniae* KPSA01 (bla_{VIM}), *Pseudomonas aeruginosa* SPM-1 (bla_{SPM}), *K. pneumoniae* KPCG01 (bla_{TEM}, $bla_{OXA\text{-}48\text{-}like}$ and bla_{NDM}), *P. aeruginosa* NTU92/99 (bla_{IMP}), and *K. pneumoniae* ATCC® BAA-1705 (bla_{KPC}) were used as a positive control in this experiment.

PCR products were purified using the purification GFX PCR™ Kit (GE Health Care, USA) and sequenced in the automated sequencer (ABI PRISM 3130XL, Applied Biosystems, USA) according to the manufacturer's recommendations. Available online BLAST software (http://blast.ncbi.nlm.nih.gov/) was used to compare the obtained sequences (Supplementary Material—Sequences) with those available in the GenBank database. Sequences of detected genes were deposited in GenBank (Table 2). The sequences alignment was performed using ChromasPro version 1.7.6 software (Technelysium Pty. Ltd.) and Clustal Omega EMBL-EMI Program.

3 Results and Discussion

In this work, three soil samples from different origins were selected to be analyzed for the presence of different types of β-lactamases-codifying genes (bla_{VEB}, bla_{PER}, bla_{TEM}, bla_{SHV}, $bla_{OXA\text{-}1}$, $bla_{CTX\text{-}M}$, $bla_{OXA\text{-}48\text{-}like}$, bla_{KPC}, bla_{AmpC}, bla_{VIM}, bla_{IMP}, bla_{SPM}, bla_{SIM}, bla_{GIM}, and bla_{NDM}). These genes have been found in several Gram-negative bacteria, including *P. aeruginosa*, *Acinetobacter baumannii*, and in well-known species of *Enterobacteriaceae*. The soil samples were obtained from a landfill, a preservation area, and from a farm. The landfill is 42 km apart from the farm, which is located 890 km apart from the Chapada dos Veadeiros National Park.

AmpC gene, which confers resistance to cephalosporin, was not detected in any sample; however, genes codifying for ESBL enzymes bla_{SHV}, $bla_{TEM\text{-}116}$, and $bla_{OXA\text{-}1}$ were found in all analyzed samples. Gene for ESBL $bla_{CTX\text{-}M\text{-}14}$ was detected in the landfill and in the farm soil samples, but they were not detected in the preservation area, while $bla_{OXA\text{-}48\text{-}like}$ was present just in the soil from the landfill. The gene for the MBL bla_{VIM} was found in the soil sample from a farm (Table 2, Fig. 1).

The results showed that the codifying genes for ESBL bla_{SHV}, $bla_{TEM\text{-}116}$, and $bla_{OXA\text{-}1}$ are spread in different areas, even in a preserved soil. The presence of

Table 1 Primers used in the study

Genes and β-lactamase(s) targeted	Primer name	Sequence (5′ - 3′)	Annealing temperature (°C)	Amplicon size (bp)	Reference
GES-1 to 9 and 11	MultiGES_for MultiGES_rev	AGTCGGCTAGACCG GAAAG TTTGTCCGTGCTCAGGAT	57	399	Dallenne et al. 2010
VEB-1 to 6	MultiVEB_for MultiVEB_rev	CATTTCCCGATGCA AAGCGT CGAAGTTTCTTTGG ACTCTG	60	648	Dallenne et al. 2010
PER-1 and 3	MultiPER_for MultiPER_rev	GCTCCGATAATGAA AGCGT TTCGGCTTGACTCG GCTGA	60	520	Dallenne et al. 2010
TEM including 1 and 2	MultiTSO-T_for MultiTSO-T_rev	CATTTCCGTGTCGC CCTTATTC CGTTCATCCATAGT TGCCTGAC	60	800	Dallenne et al. 2010
SHV including 1	MultiTSO-S_for MultiTSO-S_rev	AGCCGCTTGAGCAA ATTAAAC ATCCCGCAGATAAA TCACCAC	60	713	Dallenne et al. 2010
OXA-1, 4, and 30	MultiTSO-O_for MultiTSO-O-rev	GGCACCAGATTCAA CTTTCAAG GACCCCAAGTTTCC TGTAAGTG	60	564	Dallenne et al. 2010
CTX-M group 1 including 1, 3, and 15	MultiCTXMGp1_for MultiCTXMGp1-2_rev	TTAGGAARTGTGCC GCTGYA CGATATCGTTGGTG GTRCCAT	57	688	Dallenne et al. 2010
CTX-M group 9 including 9 and 14	MultiCTXMGp9_for MultiCTXMGp9_rev	TCAAGCCTGCCGAT CTGGT TGATTCTCGCCGCTGAAG	57	561	Dallenne et al. 2010
OXA-48-*like*	MultiOXA-48-for MultiOXA-48_rev	GCTTGATCGCCCTCGATT GATTTGCTCCGTGG CCGAAA	57	281	Dallenne et al. 2010
KPC-1 to 5	MultiKPC_for MultiKPC_rev	CATTCAAGGGCTTT CTTGCTGC ACGACGGCATAGTC ATTTGC	60	538	Dallenne et al. 2010
AmpC	PreAmpC-PA1 PostAmpC-PA2	ATGCAGCCAACGAC AAAAGG CGCCCTCGCGAGCG CGCTTC	64	1243	Rafiee et al. 2014
VIM including 1	VIM2004A VIM2004B	GTTTGGTCGCATAT CGCAAC AATGCGCAGCACCA GGATAG	58	382	Pitout et al. 2005
IMP including 1	IMP-A IMP-B	GAAGGCGTTTATGT TCATAC GTACGTTTCAAGAG TGATGC	54	587	Pitout et al. 2005
SPM-1	SPM F SPM R	CCTACAATCTAACG GCGACC TCGCCGTGTCCAGG TATAAC	55	648	Gales et al. 2003
SIM-1	SIM-F SIM-R	TACAAGGGATTCGGCATC TAATGGGGCCTGTT CCCATGTG	52	570	Ellington et al. 2007

Table 1 (continued)

Genes and β-lactamase(s) targeted	Primer name	Sequence (5′ - 3′)	Annealing temperature (°C)	Amplicon size (bp)	Reference
GIM-1	GIM-F GIM-R	TCGACACACCTTGG TCTGAA AACTTCCAACTTTG CCATGC	52	477	Ellington et al. 2007
NDM-1	NDM-F1 NDM-R1	GCAGCTTGTCGGCC ATGCGGGC GGTCGCGAAGCTGA GCACCGCAT	60	782	Peirano et al. 2011

Y = T or C; R = A or G

these genes is sufficient to confer resistance to penicillin, cephalosporins, and monobactams. Genes $bla_{\text{OXA-48-}like}$ and bla_{VIM}, which can hydrolyze carbapenems, were also detected. The first one, $bla_{\text{OXA-48-}like}$, was found just in the landfill and bla_{VIM} was present in the farm soil; however, these genes were not found in the preservation area. Therefore, it is possible to verify that the most contaminated sources have additionally the genes $bla_{\text{oxa-48}}$ and bla_{VIM}, but even the preservation area possesses resistance genes to the most of the β-lactams, which are usually used in the treatment of different infections in the medical clinical.

There are some works about the research of β-lactamases genes in soil samples (Graham et al. 2016; Gudeta et al. 2015; Pitondo-Silva et al. 2016). Graham et al. (2016) found the presence of bla_{TEM}, bla_{SHV}, bla_{OXA}, and $bla_{\text{CTX-M}}$ in the soil from Denmark and suggested that the presence of the resistance genes in animal manure and humans are interconnected and that found genes can be reduced by prudent antibiotic stewardship. Similar results were obtained by Gatica et al. (2015), who detected $bla_{\text{CTX-M-15}}$ in the sandy loam soil microcosms, $bla_{\text{OXA-1}}$ in the Hawaiian soil, and bla_{TEM} in both soil samples; however, bla_{VIM} and bla_{NDM} genes were not detected in clay soils or in the sandy loam analyzed. Pitondo-Silva et al. (2016) researched MBLs in Gram-positive isolates from soil samples in Brazil and demonstrated the prevalence of the $bla_{\text{VIM-1}}$ gene, which was detected in 19 from 40 soil samples obtained from different states and cities of that country and Pitondo-Silva et al. (2015) found bla_{VIM} gene in isolates of *Butiauxella* sp.

Different works have also shown the presence of the variant $bla_{\text{CTX-M-14}}$ in clinical and animal *E. coli* isolates (Kim et al. 2016; Liu et al. 2016; Wang et al. 2016). Variant $bla_{\text{TEM-116}}$, which was originally isolated from a

Table 2 Distribution of *bla* genes in three soil samples and the GenBank accession numbers

Sample	Source/State	Found genes	Access number
S1	Landfill/SP	$bla_{\text{CTX-M-14}}$	KX641701
		bla_{SHV}	KX641702
		$bla_{\text{TEM-116}}$	KX641703
		$bla_{\text{OXA-1}}$	KX641704
		$bla_{\text{OXA-48-}like}$	KX641705
S2	Farm/SP	$bla_{\text{CTX-M-14}}$	KX641706
		bla_{SHV}	KX641707
		$bla_{\text{TEM-116}}$	KX641708
		$bla_{\text{OXA-1}}$	KX641709
		bla_{VIM}	KX641710
S3	Enviromental reserve/ GO	bla_{SHV}	KX641711
		$bla_{\text{TEM-116}}$	KY316377
		$bla_{\text{OXA-1}}$	KX641712

São Paulo, *SP*; Goiás, *GO*

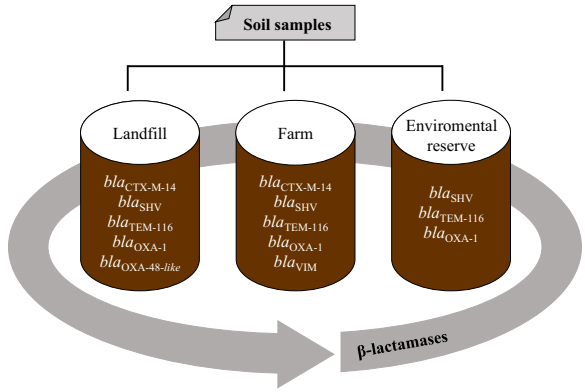

Fig. 1 Distribution of β-lactamases encoding genes in soil samples with different degrees of contamination

Staphylococcus aureus isolate, was detected in species of *Shigella flexneri* from chicken in China (Hu et al. 2007) and also in two works in Brazil, which analyzed *Aeromonas hydrophila* and *Aeromonas jandae* from water (Balsalobre et al. 2010) and clinical *K. pneumoniae* (Dropa et al. 2010). As showed by Bush and Jacoby (2016), $bla_{\text{TEM-116}}$ probably is a product of a gene cloned in the Taq production and, for this reason, we cannot consider this result as relevant.

4 Conclusions

The results indicate that some ESBL genes, as bla_{SHV} *and* $bla_{\text{OXA-1}}$, are scattered in the soils, even without potential pollutants, probably due to the fact that ESBL genes are transmissible, been carried by integrons and plasmids. The presence of these genes is sufficient to confer resistance to important β-lactams, including penicillin, cephalosporins, and monobactams, which are used in the medical clinic for the treatment of different infections. Therefore, environmental bacteria can act a reservoir of these genes and become the source of β-lactams resistant genes. Thus, this phenomenon may have clinical implications.

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Compliance with Ethical Standards

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Conflict of Interest The authors declare that they have no conflict of interest.

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