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ORIGINAL ARTICLE

PREVALENCE AND DISTRIBUTION OF RESISTANT GENES OF ANTIBIOTICS, HEAVY METALS AND BIOCIDES IN SOME *ENTEROBACTERIA* SPECIES

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Summary

The problem of bacterial resistance to antibiotics has become a global issue and a major health challenge that requires continuous studies on how this resistance develops and spreads, and its relationship to resistance to other factors such as heavy metals and biocides. The current study aimed to determine the prevalence and distribution of antibiotics, heavy metals, and biocides-resistant genes on the chromosomes and plasmids of some *Enterobacteria* species. The results showed that antibiotics resistant genes (bla_{CTX}, *sul 1*) were present in all isolates except for *Klebsiella pneumoniae* chromosome, while for heavy metals resistant genes, *czcA* detected in all isolates except for *K. pneumoniae* plasmid, ncc gene was only detected in the chromosome of *Escherichia coli* O157.H7 and *E. coli*, and plasmid of *E. coli* O157.H7. biocides gene (*qacEΔ1*) was present in all isolates except for the *E. faecalis* chromosome. The current study resulted that the studied resistance genes spread clearly among the types of *Enterobacteria*, and this reflects the possibility of transmission of these genes among the bacteria present in this habitat.

Key words: Antibiotics; Heavy metals; Biocides; Resistance genes; Enterobacteria

Introduction

Antimicrobial resistance is a global issue that makes many aspects of contemporary medicines less effective and makes it difficult to treat bacterial infections (1). Resistant strains of enteric bacteria have been associated with a large number of infections in recent decades, they have acquired resistance due to mutations in chromosomal genes and the acquisition of antibiotic resistance genes (ARGs) from other strains of the same or different species in a process called horizontal gene transfer (HGT), which It can occur in any environment, particularly where bacterial loads are high, for example in the gut microbiome of humans and animals based on the transfer-related genes carried on plasmids (2).

The primary cause of intestinal ARG formation is the unreasonable use of medical antibiotics, and prolonged clinical antibiotic use increases the abundance of the relevant intestinal ARGs (3). A combination of antibiotic-independent factors, such as heavy metals, biocides, and organic chemicals, can have an impact on antibiotic

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resistance in the environment. Such chemicals cause the emergence of antibiotic-resistant bacteria through several different mechanisms including co-resistance (where genetic determinants of tolerance are located on the same genetic component), and cross-resistance (where the same genetic determinant confers resistance to each of antibiotics, and metals/biocides), and co-regulation (where BRGs or MRGs share the same regulatory system with antibiotic-carrying genes (4).

Resistance genes reside on both chromosomes and MGEs and can therefore be transmitted between bacteria through the HGT process (5). Some of the resistance genes commonly found in enteric bacteria are antibiotic resistance genes (bla_{CTX}, bal_{SHV}, *sul1*, *qnrA*, and *qepA*, and many others), metal resistance genes (*cnrCBA*, *czcCBA*, *ncc* genes), and biocidal resistance genes (*qacA/ B*, *qacE*, *qacEΔ1*, *qacG*, and *qacF*). In addition, the presence of some ARGs such as *qnrA*, *sul1*, *tetA*, *tetM*, and *qepA* has a progressive association with heavy metals such as Zn, and Hg (6-9). The current study aimed to investigate the resistant genes of antibiotics, heavy metals, and biocides, and to determine their prevalence and distribution on the chromosomes and plasmids of some *Enterobacteria*.

Materials and methods

Studied *Enterobacteria* species: Five multidrug-resistant *Enterobacteria* species previously diagnosed in the Biology Dept. College of Science, University of Mosul, was used in this study. They included *E. coli* O157.H7, *E. coli*, *K. pneumonia*, *S. marcescens* and *Enterococcus faecalis*.

DNA extraction: Total DNA from isolates under study was extracted using Presto Mini gDNA Bacteria Kit (GT Buffer, GB Buffer, Wash1 Buffer, Wash Buffer with Ethanol, Lysozyme, Proteinase with Deionized Sterile Distilled Water, GD Columns, Collection Tubes) provided by Geneaid / Thailand, according to the manufacturer's instructions. The concentration and purity of the DNA were determined by a Nanodrop device provided by BioDrop/ English. The presence of DNA in each sample was detected by electrophoresis in 0.9% agarose gel.

Plasmid extraction: The plasmids were extracted from all studied bacteria with the Promega PureYield plasmid Miniprep system (Cell Lysis Buffer, Column Wash Solution, Elution Buffer, Endotoxin Removal Wash, Neutralization Solution, Pure yield Collection Tubes, Pure yield Minicolumns) provided by Promega/ USA, following the manufacturer's instructions. The concentration and purity of the plasmids were determined by a Nanodrop device provided by BioDrop/ English. The presence and size of plasmids in each sample were detected by electrophoresis in 0.9% agarose gel.

Detection of ARGs, HMRGs, and BRGs: The presence of ARGs, HMRGs, and BRGs on chromosomal DNA and plasmids was tested using gene-specific primers by PCR technique. Genes targeted in the current study include ARGs (bla_{CTX}, *sul1*), HMRGs (*czcA, ncc*) and biocides (*qacEΔ1*) genes using primers and PCR conditions shown in Table 1. Each PCR mix (20 µL) contained (5 µL) of sample DNA, (1 µL) of each primer, 10 µL of PCR master mix solution (Qiagen), and (3 µL) of distilled water. DNA amplification was carried out in a thermocycler (Sensoquest/Germany) under the conditions shown in Table 2. PCR-generated products were detected by electrophoresis of (15 µL) of each amplification mixture in 0.9% agarose gel in 1% Tris Borate-EDTA buffer and 0.5 µg/mL ethidium bromide.

Table 1. Primers used in the current study.

Target genes		primers Sequence (5-3)	Amplicon size (bp)	Ref.
bla_{CTX}	f	ATGTGCAGYACCAGTAARGTKATGGC	593	(10)
	r	TGGGTRAARTARGTSACCAGAAYSAGCGG		
sul 1	f	CGGCGTGGGCTACCTGAACG	433	(6)
	r	GCCGATCGCGTGAAGTTCCG		
czcA	f	GGS GCG MTS GAY TTC GGC	252	(11)
	r	GCC ATY GGN YGG AAC AT		
ncc	f	GCGTGGAAGGCAAGATGTTC	457	(11)
	r	ACGTCCACCAACGTTGGC		
qacEΔ1	f	GGCTTTACTAAGCTTGCCCC	~220	(12)
	r	AGCCCCATACCTACAAAGCC		

Table 2. PCR conditions used in the current study.

Genes	Initial denaturation	Denaturation	Annealing	extension	Final extension
bla$_{CTX}$	94 °C, 1 cycle, 5min	94 °C, 35 cycles, 30s	60 °C, 35 cycles, 15s	72 °C, 35 cycles, 30s	72 °C, 1 cycle, 5min
sul 1	95 °C, 1 cycle, 5min	95 °C, 30 cycles, 30s	55 °C,30 cycles, 30s	72 °C,30 cycles,45s	72 °C, 1 cycle, 5min
czcA	94 °C, 1 cycle, 5min	94 °C, 30 cycles, 30s	55 °C, 30 cycles, 30s	72 °C, 30 cycles, 45s	72 °C, 1 cycle, 10min
ncc	94 °C, 1 cycle, 5min	94 °C, 30 cycles, 30s	54 °C, 30 cycles, 30s	72 °C, 30 cycles, 45s	72 °C, 1 cycle, 10min
qacEΔ1	94 °C, 1 cycle, 10min	94 °C, 30 cycles,1min	57 °C, 30 cycles, 30s	72 °C, 30 cycles, 1min	72 °C, 1 cycle, 7min

Results

In this study, the resistance genes, ARGs (*bla$_{CTX}$, sul1*), HMRGs (*czcA, ncc*), and BRG (*qacEΔ1*) were detected on the chromosomes and plasmids of some *Enterobacteria* species. It is noticed from Table 3, and Figure 1, the high prevalence and distribution of these genes in studied bacteria.

The results showed that ARGs (*bla$_{CTX}$, and sul1*) genes were present in all isolates except for the *K. pneumoniae* chromosome, while for HMRGs, *czcA* detected in all isolates except for *K. pneumoniae* plasmid, *ncc* gene was only detected in chromosome and plasmid of *E. coli* O157.H7 and chromosome of *E. coli*. BRG (*qacEΔ1*) gene was present in all isolates except for the E. faecalis chromosome.

Table 3. Results of tested resistance genes detected in the studied *Enterobacteria* species.

Isolates	Chromosome					Plasmid				
	bla$_{CTX}$	*sul 1*	*czcA*	*ncc*	*qacEΔ1*	*bla$_{CTX}$*	*sul 1*	*czcA*	*ncc*	*qacEΔ1*
E.coli O157.H7	+	+	+	+	+	+	+	+	+	+
E.coli	+	+	+	+	+	+	+	+	-	+
K. pneumoniae	+	-	+	-	+	+	+	-	-	+
S. marcescens	+	+	+	-	+	+	+	+	-	+
E. faecalis	+	+	+	-	-	+	+	+	-	+
%	**100**	**80**	**100**	**40**	**80**	**100**	**100**	**80**	**20**	**100**

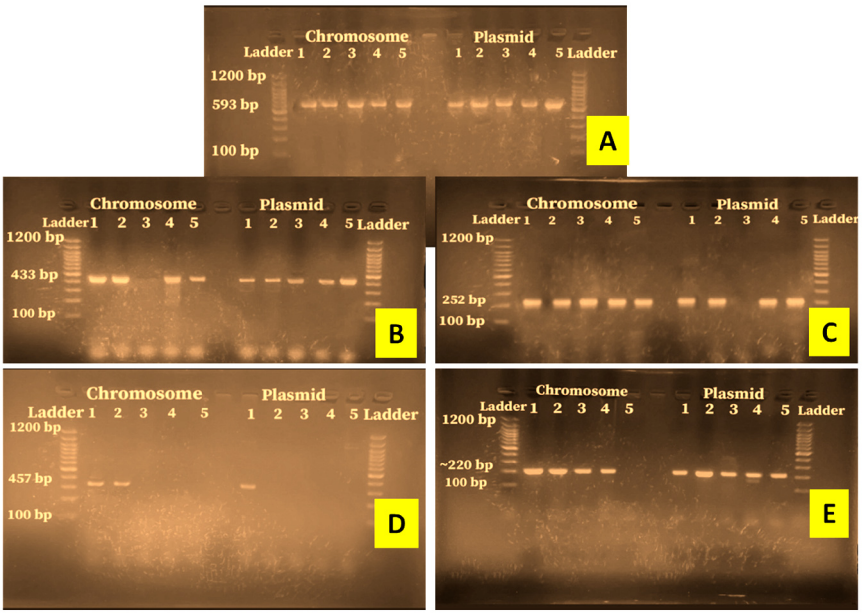

Figure 1. RT-PCR-based Gene detection in *E. coli* O157.H7, *E. coli*, *K. pneumoniae*, *S. marcescens*, and *E. faecalis* respectively from 1 to 5. (A) *bla$_{CTX}$* gene , (B) *sul1* gene , (C) *czcA* gene , (D) *ncc* gene , and (E) *qacEΔ1* gene.

Discussion

Enterobacteria often live as normal flora in the intestines of humans and animals, but some individuals have been associated with infections, especially drug-resistant strains (2). A long time ago, antimicrobials have been used to treat or prevent bacterial infections, but wrong and inappropriate use has led to the emergence of AMR among bacteria. Often, the presence of specific genetically encoded mechanisms, such as blocking access to targets, mutational alterations in antibiotic targets, and lastly modification of targets, contribute to bacterial capability to resist antimicrobials (13).

Genetic determinants (resistance genes) may be transmitted vertically or horizontally, but the most dangerous and effective is the horizontal genetic transfer (HGT) because it leads to the spread of genes easily between different strains through plasmids and transposons and turns bacteria from sensitive to resistant (14).

The results of this study showed that all isolates (100%) were positive for ARGs, and bla_{CTX} gene, and also a high percentage of *sul1* gene (100% at plasmid and 80% at chromosome) was recorded. This reflects the widespread of these genes and thus the resistance they encode, especially those carried on plasmids, which are the main factors for the horizontal gene transfer between different bacterial species. Several previous studies were consistent with our results (15-16).

Regarding HMRGs, the results showed a high incidence of the *czcA* gene (100% at chromosome and 80% at plasmid) compared to the low incidence of the *ncc* gene (40% at chromosome and 20% at plasmid). Bacteria that are exposed to heavy metals must alter their physiology and genetic makeup to survive. As a result, bacteria may acquire heavy metal resistance genes (HMRGs). The HMRG can be found on bacterial plasmids as well as chromosomes (11). Several other studies have confirmed the presence of heavy metal resistance genes carried either on chromosomes or plasmids of *Enterobacteria*, in variable percentages (11, 17).

Also, the results showed the high prevalence of the *qacEΔ1* gene among the enteric bacteria under study. Selective pressure caused by the widespread use of QACs may cause the dissemination of *qac* genes among bacteria in different niches, in addition to co-selection for BRGs by ARGs and MRGs also facilitate dissemination (18). A previous study (9) confirmed the presence of *qacEΔ1* among studied *Enterobacteriaceae* isolates, while was not observed in *E. faecalis*. another study (19) recorded the presence of *qacEΔ1* gene in all studied *E. coli* isolates.

Results of the current study confirm that the ARGs, HMRGs, and BRGs genes spread clearly among *Enterobacteria* and this reflects the possibility of transmission of these genes among the bacteria present in this habitat.

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Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

This article does not contain any studies involving animals performed by any of the authors. This article does not contain any studies involving human participants performed by any of the authors.

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