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Letter to the Editor

# Investigation the frequency of antibiotic resistance genes in drinking water samples by polymerase chain reaction in Kermanshah province

*Dear Editor,*

Since the first alarm of antibiotic resistance, this phenomenon is increasing dramatically. Therefore, with better understanding of the origin of antibiotic resistance, it is possible to prevent the entry of pharmaceuticals, chemicals, and healthcare products in sewage treatment plants, surface water, underground water, and especially drinking water. The presence and high concentration of antibiotics in the environment, especially in drinking water, leads to the release of genes and increasing the presence of Antibiotic Resistance Gene (ARG) as an emerging pollutant, which is a worrying issue. The more important issue is that, water treatment processes not only fail to remove ARGs, rather, they increase antibiotic resistance genes in environmental sources, especially water sources. Therefore, the content of ARGs in urban water systems is a serious threat to public health [1,2]. The information gained from this study shows frequency of antibiotic resistance genes in drinking water samples. In this study, 42 drinking water samples from the main urban water sources were collected and filtered (47 mm membrane filters (filters (Cellulose Nitrate filter, Sartorius Stedium Biotech, GmbH, Göttingen Germany) and using a vacuum pump with pores of 0.45 µm (JB vacuum pump 2.5 $m^3$/hrs, American) and filter holder system (filter holder 25mm Sartorius, Germany)). After culture and DNA extraction (QIAamp DNA extraction kit (Qiagen, Valencia, USA), PCR assay PCR (C1000 Touch Thermal Cycler, Bio-Rad, Singapore) using specific primers (Table 1).

Finally, the PCR products were analyzed using electrophoresis on 1% agarose gel. The results of our study determined that the highest and lowest frequencies were related to *CTXM-1* gene 36 (85%) and *vanB*, and *NDM* 2 (4.7%). The frequencies of *IMP*, *gnrB*, *mecA*, and *vanA* genes were 18 (42.8%), 11 (26.1%), 8 (19%), and 4 (9.52%), respectively. Other genes were not isolated. BLAST sequencing results showed full similarity (99–100%). as we know, one of the main causes of drinking water pollution is the widespread use and introduction of antibiotics in healthcare and the human excretory system. We want to emphasize the necessity of conducting more research to identify antibiotic resistance genes, especially beta-lactam and carbapenem genes in drinking water. Unfortunately, the frequency of *CTXM-1* and *IMP* genes was significant in our studied samples. This increase prevalence in outside medical and health centers is alarming. The simultaneous presence of all genes in 39 samples (92.8%) of drinking water is very worrying. In the study by Lyimo et al. [3] *CTXM-1* showed the highest frequency in drinking water, while the study by Zhang et al. [4] showed a different result. The important point is that pollution in drinking water distribution networks is due to the use of contaminated water sources and the entry of human pathogens into water sources through hospital and domestic sewage. The high frequency of *CTXM-1* gene proves the high prevalence of ARGs in hospital and city water networks. *TEM* and *SHV* genes are among the most important ESBL genes that their presence in environmental samples including drinking water is low and fortunately in our study, *SHV-1*, *TEM* and *VIM* genes were not found in any sample. The study by Jiang et al. [5] is consistent with our study regarding the absence of the *SHV* gene. In fact, the high frequency of antibiotic resistance genes can be related to the presence of some colonized pathogenic bacteria in humans. In addition, some bacteria are opportunistic pathogens in humans that can affect the microbial composition of the gut and by interacting with resistant bacterial species, affect the body flora microorganisms and cause various infections in humans. on the other hand, the high prevalence of antibiotic resistance genes can be related to the pattern of multiple drug resistance of bacteria in drinking water.

**Table 1**
Research primers.

| Primer | Sequence Primer (5 → 3) | Product size (bp) |
|---|---|---|
| *vanA* | F:TCGTTGACATACATCGTTGC R: CACCGAAGGATGAGCCTG | 142 bp |
| *vanB* | F: GGTGCGATACAGGGTCTG R:CTCAACCGGATTTGATCCAC | 395 bp |
| *MecA* | F: ACCACTTCATATCTTGTAACG R: AGATTACAACTTCACCAGGTTC | 160 bp |
| *IMP* | F: GCTGAGGCTTATCTAATTGAC R: CCAACTTCACTGTGACTTGG | 479 bp |
| *VIM* | F: GCAGTCTCCACGCACTTTC R: CTCGATGAGAGTCCTTCTAG | 155 bp |
| *TEM* | F: TAAAATTCTTGAAGACG R:TTACCAATGCTTAATCA | 1074 bp |
| *NDM* | F: GACTTATGCCAATGCGTTGTC R: GCTCATCACGATCATGCTG | 334 bp |
| *SHV-1* | F: ATGCGTTATATTCGCCTGTGT R: TTGCCAGTGCTCGTACAGC | 855 bp |

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## CRediT authorship contribution statement

**Sepide Kadivarian:** Writing – original draft. **Ramin Abiri:** Writing – review & editing. **Hasti Zahedi:** Investigation. **Farhad Babaei:** Data curation, Conceptualization, Data curation, Project administration. **Amirhooshang Alvandi:** Conceptualization, Data curation, Project administration.

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## Declaration of competing interest

The authors have declared that no competing interests exist.

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