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Original Article

Biofilm formation and antibiotic resistance profiles of water-borne pathogens

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ABSTRACT

Water sources (surface water, drinking water, rivers, and ponds) are significant reservoirs for transmitting antibiotic-resistant bacteria. In addition, these waters are an important public health problem because they are suitable environments for transferring antibiotic resistance genes between bacterial species. Our study aimed to assess the prevalence of Extended-spectrum beta-lactamase (ESBL) producing isolates in water samples, the susceptibility of the isolates to the specified antibiotics, the determination of biofilm ability, antibiotic resistance genes, and the molecular typing of the isolates. For this purpose, Polymerase chain reaction (PCR) and Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) analyses were used. Out of 70 isolates, 15 (21%) were ESBL producing, and sent for the MALDI-TOF analysis, where *Escherichia coli, Acinetobacter calcoaceticus, Enterobacter bugandensis, Acinetobacter pittii, Pseudomonas aeruginosa, Acinetobacter junii, Pseudomonas oleovorans,* and *Enterobacter ludwigigii* were identified. Moreover, colistin resistance genes (*mcr 1/2/6, mcr 4, mcr 5, mcr 3/7*, and *mcr 8*), ESBL-encoding genes (bla_{SHV}, bla_{TEM}, and bla_{CTX-M}) and carbapenemase genes (bla_{NDM}, bla_{OXA-48}, and bla_{KPC}) using molecular analysis (PCR) were confirmed. The colistin resistance gene was detected at 80% (12/15) in the isolates obtained. The distribution of these isolates according to resistance genes was found as *mcr 1/2/6* 4 (20%), *mcr 3/7* 3 (13%), and *mcr 5* (40%). Additionally, the isolates harbored bla_{SHV}(6.6%) and bla_{TEM} (6.6%) genes. However, bla_{NDM}, bla_{OXA-48}, bla_{KPC}, and bla_{CTX-M} genes were not detected in any isolates. According to the Congo red agar method, seven (46.6%) isolates showed negative biofilm ability, and eight (53.3%) showed moderate biofilm ability. However, the microplate method detected weak biofilm in 53.3% of the isolates. In conclusion, this study provides evidence for the existence of multidrug-resistant bacteria that co-exist with *mcr* and ESBL genes in water sources. These bacteria can migrate to other environments and pose increasing threats to public health.

1. Introduction

Studies show that 10 million people will die annually by 2050 due to increased in antibiotic resistance, and an economic loss of 100 trillion US dollars will be experienced [1]. The unconscious or uncontrolled use of antibiotics is recognized as essential to the emergence and spread of antibiotic-resistant pathogenic bacteria [2,3]. Antibiotics are widely used to promote weight gain in food-producing animals, control zoonotic pathogens, and treat bacterial and fungal diseases in humans and animals [4]. Antibiotics are scarcely absorbed in human and animal guts, with their generality being excreted unchanged in feces and urine [5]. They are eventually released into the environment through sewage water and wastewater [6,7]. However, the ability of antibiotic-resistant bacteria to survive in the aquatic environment and their resistance genes are not fully understood. Antibiotic-resistant Gram-negative bacteria are favored as the subject of investigation because many species are native inhabitants of water environments. Colistin resistance bacteria have shown omnipresence (e.g., in the environment, food, water, and plants) in the last decade [8]. Another risk in pathogens bacteria is biofilm-forming ability. Biofilms are defined as clusters of microorganisms that adhere to a specific surface and are surrounded by extracellular matrix components. Its biofilm-forming ability protects pathogenic microorganisms from environmental factors such as heat, detergents, and inhibitors. In addition to facilitating cellular connectivity and horizontal gene transfer, biofilms provide definitive protection for the bacterial community by complicating the diffusion of antimicrobials through the matrix [9]. Bacteria with biofilm properties continue to spread in waters, creating a potential epidemic risk [10,11]. The present study aims to determine the antibiotic resistance profiles, antibiotic resistance gene distribution, and biofilm formation abilities of isolates isolated from water sources.

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2. Material and method

2.1. Sampling

Between 2020 and 2022, a total of 300 water samples were collected from various water bodies, including surface water (n = 113), pools (n = 77), and drinking water (n = 110). The water samples were randomly taken each month, and the sampling sites were approximately 200 m apart. From each site, samples were collected in sterile 500 ml containers and transferred to the laboratory in a cool box on the same day.

2.2. Isolation of bacteria

Bacteria were obtained using the standard membrane filter procedure outlined in SM 9222 [12]. In brief, water samples (both undiluted and diluted) were filtered through sterile 0.45 μm polyethersulfone membranes (Mo Bio Laboratories, Carlsbad, CA, USA), and the membranes were then inoculated onto Chromogenic™ CCA (Merck, Germany) agar plates supplemented with cefotaxime (4 μg/ml). They were incubated at 37 °C for 24 h, and all resulting colonies were received. All isolates were sent to Hatay Mustafa Kemal University for matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF) analysis to identify the bacterial species.

2.3. Antimicrobial susceptibility testing

The current study used the disk diffusion method according to the Clinical and Laboratory Standards Institute [13] to evaluate the results of eleven antimicrobial agents: piperacillin/tazobactam, amoxicillin/clavulanic acid, gentamicin, cefotaxime, cefoxitin, amikacin, ciprofloxacin, cefazolin, ceftazidime, meropenem, and sulfamethoxazole/trimethoprim. ESBL-producing isolates were evaluated using the double disk synergism (DDS) method with cefotaxime and ceftazidime plus amoxicillin/clavulanic acid (Table 3). The minimum inhibitory concentration (MIC) for Colistin of each isolate was determined using the broth microdilution (BMD) method following the CLSI procedure.

2.4. DNA extraction process

DNA extraction was performed on each isolate using the instaGene Matrix (Bio-Rad, USA), and the extracted DNA was used for all subsequent PCRs.

Table 1
Primers used in the study and PCR amplification conditions.

Genes	Forward	References	Bp	PCR amlification conditions
trpA F *trpA*R	CGGCGATAAAGACATCTTCAC GCAACGCGGCCTGGCGGAAG	Clermont et al. [14]	489	94ºC for 2 min 94ºC for 20 sn 54ºC for 1 min 72ºC for 1 min (35 cycling) 72ºC for 10 min
P8 F *P8* R	CGTGATGATGTTGAGTTG AGATTGGTTGGCATTACTG	Sarimehmetoglu et al. [15]	400	94°C for 5 min 94°C for 1 min 53°C for 1 min 72°C for 1 min (35 cycling) 72°C for 10 min
Bla *KPC* F Bla *KPC* R Bla *NDM* F Bla *NDM* R Bla *OXA48* F Bla *OXA48* R	CGTCTAGTTCTGCTGTCTTG CTTGTCATCCTTGTTAGGCG GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC GCGTGGTTAAGGATGAACAC CATCAAGTTCAACCCAACCG	Poirel et al. [16]	798 621 438 	94°C for 5 min 94°C for 1 min 56.4°C for 1 min 72°C for 1 min (35 cycling) 72°C for 10 min
Bla *SHV* F Bla *SHV* R Bla *TEM* F Bla *TEM* R Bla *CTX-M* F Bla*CTX*-MR	TCGGGCCGCGTAGGCATGAT AGCAGGGCGACAATCCCGCG ATAAAATTCTTGAAGACGAAA GACAGTTACCAATGCTTAATCA ACGCTGTTGTTAGGAAGTG TTGAGGCTGGGTGAAGT	Gundran et al. [17]	626 1080 759 	94°C for 5 min 94°C for 1 min 58°C for 1 min 72°C for 1 min (30 cycling) 72°C for 10 min
mcr1/2/6 F *mcr1/2/6* R *mcr3/7*R *mcr3/7*F *mcr 4* F *mcr 4* R *mcr 5* F *mcr 5* R *mcr 8* F *mcr 8* R	GTCGTCGGTGAGACGGC GTATTTGGCGGTATCGACATCA AACACATGCTATGACGAGTTT GGTGTAGCGGATGGTGTTGTC TGCGAAGAATGCCAGTCGTA GCCGCATGAGCTAGTATCGT TGCGCAACTACGGGGTTTAT CGAATGCCCGAGATGACGTA CCTGCATGTTCTCGCGAATG GCATCCCGGAATAACGTTGC	Wang et al. [18]	198 228 169 328 486 	94°C for 30 sn 94°C for 15 sn 65°C for 1 min 72°C for 1 min (40 cycling)

2.5. Confirmation of Escherichia coli

DNA samples of *E. coli* were analyzed using PCR with DreamTaq Hot Start PCR (2*x*) (Thermo Fisher, USA) following the manufacturer's instructions. PCR amplification conditions are provided in Table 1.

2.6. PCR analysis of antimicrobial resistance genes

PCR was used to detect genes related to ESBL (bla_{SHV}, bla_{TEM}, and bla_{CTX-M}) and carbapenemase genes (bla_{NDM}, bla_{OXA-48}, and bla_{KPC}). DreamTaq Hot Start PCR (2*x*) from Thermo Fisher (USA) was used for the PCR analyses. The resulting PCR products were evaluated by electrophoresis on a 1.5% agarose gel containing ethidium bromide (0.1 μg/ml) in 1*X* TBE buffer. Electrophoresis was carried out at 100 V for 45 min, and the DNA bands were visualized with an ultraviolet transilluminator from Vilber Lourmat (Marne La Vallee, France). The primers and amplification details are shown in Table 1.

2.7. Colistin genes identification withReal-Time PCR

Colistin resistance genes were confirmed using the Sybr Green (Bio-Rad, USA) procedure with Real-Time PCR. The primers used were *mcr 1/2/6*, *mcr 3/7*, *mcr 4*, *mcr 5*, and *mcr 8*. PCR amplification conditions and primers are given in Table 1.

2.8. Determination of biofilm characteristics of isolates

Fifteen isolates were inoculated on Congo Red Agar (Merck, Germany) and incubated for 24–48 h at 37 °C. The colonies were evaluated as having strong, weak, and negative biofilm-forming capacities based on their color (black, dark-brown, and pinkish-red, respectively) [19,20]. Biofilm formation was measured at a mean absorbance of 570 nm (ODc). The biofilm-forming ability of the isolates was qualified as follows: OD $\leq$ ODc for biofilm-negative, ODc < OD $\leq$ 2*X* ODc for weak biofilm, 2*X* ODc < OD $\leq$ 4*X* ODc for moderate biofilm, and OD > 4*X* ODc for strong biofilm formation using the microplate method [21]. The positive control used was the *E. coli* ATCC 25922 strain.

3. Results

In this study, 70 isolates were inoculated on Chromogenic™ CCA (Merck, Germany) agar plates supplemented with cefotaxime (4 μg/ml), afterward 15 (21%) isolates obtained were identified using the MALDI-TOF. The analysis showed that 40% of the isolates were *E. coli* and 6.6% *Acinetobacter calcoaceticus, Enterobacter bugandensis, Acinetobacter pittii, Pseudomonas aeruginosa, Acinetobacter junii, and Pseudomonas oleovorans,* and *Enterobacter ludwigigii* were identified. The Antibiotic susceptibility with disc diffusion testing was identified. According to testing, isolates showed resistance to CZ 12 (80%), FOX 8 (53%), CTX 7 (47%), SXT 7 (47%), AMC 6 (40%), CIP 5 (33%), CN 2 (27%), TPZ1 (6%) while the isolates showed susceptible to TPZ 7 (47%), AMC 6 (40%), CN 12 (67%), FOX 7 (47%), AK 15 (100%), CIP 10 (67%), CZ 1 (7%), CAZ 13 (87%), MEM 15 (100%), SXT 8 (53%), and CTX 3 (10%). The results also showed that the isolates have multi-drug resistance (Tables 4

Table 3
Antibiotics resistance profiles distributed of isolates.

Name of Antibiotics	S	I	R
TPZ	7 (47%)	7 (47%)	1 (6%)
AMC	6 (40%)	3 (20%)	6 (40%)
CN	12 (67%)	1 (6%)	2 (27%)
FOX	7 (47%)	–	8 (53%)
AK	15 (100%)	–	–
CIP	10 (67%)	–	5 (33%)
CZ	1 (7%)	2 (13%)	12 (80%)
CAZ	13 (87%)	–	2 (13%)
MEM	15 (100%)	–	–
SXT	8 (53%)	–	7 (47%)
CTX	3 (10%)	5 (33%)	7 (47%)

S: Susceptible; I: Intermediate; R: Resistance, TPZ: Piperacillin/tazobactam, AMC: Amoxicillin/clavulanic acid; CN: Gentamicin; FOX: Cefoxitin, AK: Amikacin, CIP, Ciprofloxacin; CZ: Cefazolin, CAZ: Ceftazidime, MEM: Meropenem; SXT: Sulphamethoxazole/trimethoprim, CTX: Cefotaxime.

Table 4
Antibiotic resistance profiles of each isolate**s**.

ID of Isolates	Name of bacteria	Antibiotics
W11	*Escherichia coli*	FOX, CIP, CZ, SXT, CTX
W12	*Escherichia coli*	FOX, CZ, SXT, CTX
W13	*Escherichia coli*	CZ
W14	*Escherichia coli*	CZ, CTX
W15	*Escherichia coli*	AMC, FOX, CZ, SXT, CTX
W3	*Acinetobacter pittii*	AMC, FOX, CZ
W2	*Enterobacter bugandensis*	CN, CIP, CZ, SXT, CTX
W6	*Pseudomonas oleovorans*	AMC, FOX, CZ
W1	*Acinetobacter calcoaceticus*	CAZ
W4	*Pseudomonas aeruginosa*	TPZ, AMC, CN, FOX, CZ, SXT, CTX, CIP
W7	*Enterobacter ludwigii*	AMC, CN, FOX, CIP, CZ, SXT, CTX
W9	*Comamonas aquatica*	AMC, FOX, CIP
W8	*Enterobacter bugandensis*	CIP, CZ, CAZ, SXT, CTX

Table 2
Antibiotic resistance gene profiles of positive samples.

ID of Isolates	*Name of Bacteria*	*mcr 1/2/6*	*mcr 3/7*	*mcr 4*	*mcr-5*	*mcr 8*	bla_{SHV}	bla_{TEM}	bla_{CTX-M}	bla_{KPC}	bla_{NDM}	bla_{OXA-48}
W1	*Acinetobacter calcoaceticus*	–	–	–	–	–	–	+	–	–	–	–
W2	*Enterobacter bugandensis*	–	+	–	+	–	–	–	–	–	–	–
W3	*Acinetobacter pittii*	+	–	–	–	–	+	–	–	–	–	–
W4	*Pseudomonas aeruginosa*	+	–	–	–	–	–	–	–	–	–	–
W5	*Acinetobacter junii*	+	–	–	–	–	–	–	–	–	–	–
W6	*Pseudomonas oleovorans*	–	–	–	+	–	–	–	–	–	–	–
W7	*Enterobacter ludwigii*	+	+	–	+	–	–	–	–	–	–	–
W8	*Enterobacter bugandensis*	–	–	–	–	–	–	–	–	–	–	–
W9	*Comamonas aquatica*	–	–	–	+	–	–	–	–	–	–	–
W10	*Escherichia coli*	–	+	–	+	–	–	–	–	–	–	–
W11	*Escherichia coli*	–	–	–	+	–	–	–	–	–	–	–
W12	*Escherichia coli*	–	–	–	–	–	–	–	–	–	–	–
W13	*Escherichia coli*	–	–	–	–	–	–	–	–	–	–	–
W14	*Escherichia coli*	–	–	–	–	–	–	–	–	–	–	–
W15	*Escherichia coli*	–	–	–	–	–	–	–	–	–	–	–

and 5). Broth microdilution testing was performed for the colistin. As a result of broth microdilution testing, most of the isolates (9/15) exhibited MIC values of MIC ≥4 μg/ml. Antimicrobial resistance genes: *mcr 1/2/6*, *mcr 4*, *mcr 5*, *mcr 3/7*, *mcr 8* (for colistin), bla_{SHV}, bla_{TEM}, bla_{CTX-M} (for beta-lactamase) and bla_{NDM}, bla_{OXA-48}, and bla_{KPC} (for carbapenemase) confirmed by molecular analyzes (mPCR and Real-Time PCR). The isolates harbored colistin resistance genes *mcr 1/2/6 4* (20%), *mcr 3/7*3 (13%), and *mcr 5* (40%). Additionally, the isolates harbored beta-lactamase bla_{SHV} (6.6%) and bla_{TEM} (6.6%). However, bla_{NDM}, bla_{OXA-48}, bla_{KPC} and bla_{CTX-M} genes were not detected in any isolates (Table 2). The Congo red agar method and the microplate method showed different results in determining the biofilm production abilities of the bacterial isolates. While the Congo red agar method indicated that 46.6% of the isolates were biofilm negative and 53.3% had moderate biofilm abilities, the microplate method showed that 53.3% of the strains had weak biofilm production and 46.6% had no biofilm production (Table 6).

4. Discussion

In this study, samples were inoculated on Chromogenic™ CCA (Merck, Germany) agar plates supplemented with cefotaxime (4 μg/ml), afterward 15 (21%) isolates were identified using the MALDI-TOF. The analysis showed that 40% of the isolates were *E. coli* and 6.6% *A. calcoaceticus, E. bugandensis, A. pittii, P. aeruginosa, A. junii, and P. oleovorans,* and *E. ludwigigii* were identified.

Acinetobacter spp. Is a globally recognized opportunistic pathogen responsible for nosocomial infections, with extreme drug resistance to many chemotherapeutics, making it an ongoing public health threat. A study by [22] reported 10.34% positivity of *A. pittii* using the MALDI-TOF analysis, while [23] found *A. junii* positive at 21.8%. Enterobacteriaceae have been detected in various environments, including wastewater, surface water, soil, and food, and have been known to play a significant role in food corruption. In a study by [24]; *Enterobacter ludwigii* was detected at a level of 11%.

Table 5
Multidrug resistance profiles of isolates.

Name of Antibiotics	Number of Positive Isolates
CN, CIP, CZ, SXT, CTX	1
FOX, CIP, CZ, SXT, CTX	1
AMC, FOX, CZ, SXT, CTX	1
AMC, CN, FOX, CIP, CZ, SXT	1
AMC, CN, FOX, CIP, CZ, SXT, CTX	1

Table 6
Biofilm-Forming ability of the obtained isolates.

ID of Isolates	Congo Red Agar	Microplate Method
W1	None-biofilm	Weak
W2	Moderate	Weak
W3	Moderate	Weak
W4	None-biofilm	Weak
W5	None-biofilm	Weak
W6	None-biofilm	Weak
W7	None-biofilm	None-biofilm
W8	None-biofilm	Weak
W9	None-biofilm	Weak
W10	Moderate	None-biofilm
W11	Moderate	None-biofilm
W12	Moderate	None-biofilm
W13	Moderate	None-biofilm
W14	Moderate	None-biofilm
W15	Moderate	None-biofilm

Pseudomonas, a diverse bacterial species known for its metabolic adaptability and genetic and physiological abilities, were found to survive in various environmental conditions including marine habitats [25]. reported 24.5% positivity of *P. aeruginosa* in drinking water. The genus *Comamonas*, assigned to Comamonadaceae, was proposed by [26] and later updated and published as *Comamonas terrigena* by [27]. [28] also isolated *Comamonas aquatilis* in pond waters.

In our study, isolates showed resistance to CZ 12 (80%), FOX 8 (53%), CTX 7 (47%), SXT 7 (47%), AMC 6 (40%), CIP 5 (33%), CN 2 (27%), TPZ 1 (6%) antibiotics [29]. reported isolates susceptible to AMP and IPM (45%), AMX (43%), and AMC (31%) antibiotics. Also, another study by [30] on antibiotic susceptibility revealed that isolates found high resistance to AMC (100%), AM (81%), and CTX (81%). Besides, the alarming rise of antibiotic resistance and the scarcity of new drugs have led to a lack of effective antibiotics. Thus, despite its toxicity, colistin has been reintroduced in human medicine as a last-resort antibiotic. The colistin resistance gene was detected in 80% (12/15) of the obtained isolates. The distribution of these isolates according to resistance genes was: *mcr 1/2/6* in 4 (20%), *mcr 3/7* in 3 (13%), and *mcr 5* in 2 (40%) of the isolates. Additionally, the isolates harbored ESBL genes bla_{SHV} (6.6%) and bla_{TEM} (6.6%). A study conducted by [31] with Enterobacteriaceae found a rate of 92.3% for bla_{TEM},bla_{CTX-M}, and bla_{SHV}. Another study stated an 11.9% rate of colistin resistance gene in *P. aeruginosa* isolates [32]. [33] reported colistin resistance genes and ESBL genes in all Enterobacteriaceae *(E. coli, K. pneumoniae, Citrobacter freundii, Citrobacter koseri, Enterobacter kobei, Enterobacter cloacae, E. ludwigii, and Enterobacter asburiae)* isolates. However, bla_{NDM}, bla_{OXA-48}, bla_{KPC}, and bla_{CTX-M} genes were not detected in any isolates in our study.

The Congo red agar is used to increase the production of exopolysaccharides, which allows for the quantitative assessment of biofilm formation in Gram negative microorganisms. In one study, 90% of the isolates showed biofilm-producing activity [34]. Using the Congo red agar method in the current study, seven isolates (46.6%) were found to be biofilm-negative, and eight isolates (53.3%) showed moderate biofilm abilities. This study determined strains eight (53.3%) weakly and seven (46.6%) non-biofilm production with the microplate method. Contrary to this study, [34]; only one *Enterobacter* strain determined moderate biofilm production detected with the microplate method.

5. Conclusion

This study aims to identify the antibiotic resistance profiles, genes, and biofilm-forming abilities of isolates from different water sources in Turkey. Given the increase in antimicrobial resistance, it is crucial to determine the importance of antibiotic-resistance genes in waters for public health, regardless of the income level of the countries. Further research is necessary to understand the risk, better characterize the problem and develop solutions to combat antimicrobial-resistant bacteria in water. Antimicrobial resistance monitoring programs in all reservoirs and strategic action plans are required to reduce antimicrobial resistance in animals and humans and encourage the prudent use of antimicrobials.

Declaration of competing interest

The authors declare no conflict of interest.

References

[1] O'Neill J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. 2016.

[2] Kumar S, Tripathi VR, Garg SK. Antibiotic resistance and genetic diversity in water-borne Enterobacteriaceae isolates from recreational and drinking water sources. Int J Environ Sci Technol 2013;10(4):789–98. https://doi.org/10.1007/s13762-012-0126-7.
[3] Asokan GV, Vanitha A. WHO global priority pathogens list on antibiotic resistance: an urgent need for action to integrate One Health data. Perspectives in public health 2018;138(2):87–8. https://doi.org/10.1177/1757913917743881.
[4] Peng M, Salaheen S, Biswas D. Animal health: global antibiotic issues. Encyclopedia of agriculture and food systems 2014:346.
[5] Pokharel S, Shrestha P, Adhikari B. Antimicrobial use in food animals and human health: time to implement 'One Health'approach. Antimicrob Resist Infect Control 2020;9:1–5.
[6] Schlüsener MP, Bester K. Persistence of antibiotics such as macrolides, tiamulin and salinomycin in soil. Environ Pollut 2006;143(3):565–71. https://doi.org/10.1016/j.envpol.2005.10.049.
[7] Tao R, Ying GG, Su HC, Zhou HW, Sidhu JP. Detection of antibiotic resistance and tetracycline resistance genes in Enterobacteriaceae isolated from the Pearl rivers in South China. Environ Pollut 2010;158(6):2101–9. https://doi.org/10.1016/j.envpol.2010.03.004.
[8] Kittinger C, Lipp M, Folli B, Kirschner A, Baumert R, Galler H, et al. Enterobacteriaceae isolated from the River Danube: antibiotic resistances, with a focus on the presence of ESBL and carbapenemases. PloS one 2016;11(11):e0165820. https://doi.org/10.1371/journal.pone.0165820.
[9] Vieira MS, da Silva JD, Ferro CG, Cunha PC, Vidigal PMP, da Silva CC, et al. A highly specific Serratia-infecting T7-like phage inhibits biofilm formation in two different genera of the Enterobacteriaceae family. Res Microbiol 2021;172(6):103869. https://doi.org/10.1016/j.resmic.2021.103869.
[10] Huq A, Whitehouse CA, Grim CJ, Alam M, Colwell RR. Biofilms in water, its role and impact in human disease transmission. Curr Opin Biotechnol 2008;19(3):244–7. https://doi.org/10.1016/j.copbio.2008.04.005.
[11] Zhang J, Li W, Chen J, Qi W, Wang F, Zhou Y. Impact of biofilm formation and detachment on the transmission of bacterial antibiotic resistance in drinking water distribution systems. Chemosphere 2018;203:368–80. https://doi.org/10.1016/j.chemosphere.2018.03.143.
[12] Baird RB, Eaton AD, Clesceri LS. In: Rice EW, editor. Standard methods for the examination of water and wastewater, vol. 10. Washington, DC: American public health association; 2012.
[13] CLSI, Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 30th informational supplement M100 ED30. PA: Wayne; 2020.
[14] Clermont O, Lavollay M, Vimont S, Deschamps C, Forestier C, Branger C, et al. The CTX-M-15-producing Escherichia coli diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. J Antimicrob Chemother 2008;61(5):1024–8. https://doi.org/10.1093/jac/dkn084.
[15] Sarimehmetoglu B, Aksoy MH, Ayaz ND, Ayaz Y, Kuplulu O, Kaplan YZ. Detection of Escherichia coli O157: H7 in ground beef using immunomagnetic separation and multiplex PCR. Food Control 2009;20(4):357–61. https://doi.org/10.1016/j.foodcont.2008.06.002.
[16] Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70(1):119–23. https://doi.org/10.1016/j.diagmicrobio.2010.12.002.
[17] Gundran RS, Cardenio PA, Salvador RT, Sison FB, Benigno CC, Kreausukon K, et al. Prevalence, antibiogram, and resistance profile of extended-spectrum β-lactamase-producing Escherichia coli isolates from pig farms in luzon, Philippines. Microb Drug Resist 2020;26(2):160–8. https://doi.org/10.1089/mdr.2019.0019.
[18] Wang X, Li J, Cao X, Wang W, Luo Y. Isolation, identification and characterisation of an emerging fish pathogen, Acinetobacter pittii, from diseased loach (Misgurnus anguillicaudatus) in China. Antonie van Leeuwenhoek 2020;113(1):21–32. https://doi.org/10.1007/s10482-019-01312-5.
[19] Handke LD, Conlon KM, Slater SR, Elbaruni S, Fitzpatrick F, Humphreys H, et al. Genetic and phenotypic analysis of biofilm phenotypic variation in multiple Staphylococcus epidermidis isolates. J Med Microbiol 2004;53(5):367–74. https://doi.org/10.1099/jmm.0.05372-0.
[20] Kreil-Ouschan C, Csokai J, Mueller E. Evaluation of Staphylococcus pseudintermedius and incidence of in vitro biofilm production in healthy dogs and dogs with pyoderma. Wiener Tierärztliche Monatsschrift 2017;104(1/2):31–4.
[21] Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods 2000;40(2):175–9. https://doi.org/10.1016/S0167-7012(00)00122-6.
[22] Topić Popović N, Kazazić S, Čož-Rakovac R. MALDI-TOF MS Profiling of Piscine Acinetobacter Spp. From Wastewater-Related Waters. 2022. https://doi.org/10.2298/VETGL211201005T.
[23] Pindi PK, Raghuveer Yadav P, Shiva Shanker A. Identification of opportunistic pathogenic bacteria in drinking water samples of different rural health centers and their clinical impacts on humans. BioMed Rest Int 2013;2013. https://doi.org/10.1155/2013/348250.
[24] Cho GS, Stein M, Fiedler G, Igbinosa EO, Koll LP, Brinks E, et al. Polyphasic study of antibiotic-resistant enterobacteria isolated from fresh produce in Germany and description of Enterobacter vonholyi sp. nov. isolated from marjoram and Enterobacter dykesii sp. nov. isolated from mung bean sprout. Syst Appl Microbiol 2021;44(1):126174. https://doi.org/10.1016/j.syapm.2020.126174.
[25] Wei L, Wu Q, Zhang J, Guo W, Gu Q, Wu H, et al. Prevalence, virulence, antimicrobial resistance, and molecular characterization of pseudomonas aeruginosa isolates from drinking water in China. Front Microbiol 2020;11:544653. https://doi.org/10.3389/fmicb.2020.544653.
[26] Davis GHG, Park RWA. A taxonomic study of certain bacteria currently classified as Vibrio species. Microbiology 1962;27(1):101–19. https://doi.org/10.1099/00221287-27-1-101.
[27] De Vos P, Kersters K, Falsen E, Pot B, Gillis M, Segers P, et al. Comamonas Davis and Park 1962 gen. nov., nom. rev. emend., and Comamonas terrigena Hugh 1962 sp. nov., nom. rev. Int J Syst Evol Microbiol 1985;35(4):443–53. https://doi.org/10.1099/00207713-35-4-443.
[28] Kämpfer P, Busse HJ, Baars S, Wilharm G, Glaeser SP. Comamonas aquatilis sp. nov., isolated from a garden pond. Int J Syst Evol Microbiol 2018;68(4):1210–4. https://doi.org/10.1099/ijsem.0.002652.
[29] Salamandane A, Vila-Boa F, Malfeito-Ferreira M, Brito L. High fecal contamination and high levels of antibiotic-resistant Enterobacteriaceae in water consumed in the city of Maputo, Mozambique. Biology 2021;10(6):558. https://doi.org/10.3390/biology10060558.
[30] Yuan W, Zhang Y, Riaz L, Yang Q, Du B, Wang R. Multiple antibiotic resistance and DNA methylation in Enterobacteriaceae isolates from different environments. J Hazard Mater 2021;402:123822. https://doi.org/10.1016/j.jhazmat.2020.123822.
[31] Ibrahim ME, Algak TB, Abbas M, Elamin BK. Emergence of bla TEM, bla CTX-M, bla SHV and bla OXA genes in multidrug-resistant Enterobacteriaceae and Acinetobacter baumannii in Saudi Arabia. Exp Ther Med 2021;22(6):1–11. https://doi.org/10.3892/etm.2021.10885.
[32] Hameed F, Khan MA, Muhammad H, Sarwar T, Bilal H, Rehman TU. Plasmid-mediated mcr-1 gene in Acinetobacter baumannii and Pseudomonas aeruginosa: first report from Pakistan. Rev Soc Bras Med Trop 2019;52.
[33] Ovejero CM, Delgado-Blas JF, Calero-Caceres W, Muniesa M, Gonzalez-Zorn B. Spread of mcr-1-carrying Enterobacteriaceae in sewage water from Spain. J Antimicrob Chemother 2017;72(4):1050–3. https://doi.org/10.1590/0037-8682-0237-2019.
[34] Ramos GLDPA, dos Santos Nascimento J. Antibiotic resistance profile and detection of degradative enzymes by Enterobacteriaceae isolated from raw goat milk. Germs 2021;11(2):211. https://doi.org/10.18683/germs.2021.1258.