Research Article
Volume 10:1, 2021

Expert Opinion on Environmental Biology

Biofilm Former and Chlorine Resistance Enterobacter cloacae in Water Storage Tanks can increase the Threat of Waterborne Disease

***Hastyar H. Najmuldeen[1 & 2]**

[1] *Department of Biology, College of Science, University of Sulaimani, Kurdistan Region, Iraq*

[2] *College of Science, Komar University of Science and Technology, Sulaimani, Kurdistan Region, Iraq*

Keywords: MPN, Chlorine treatment and resistant, Antibiotic resistant, Water storage tanks, Enterobacter cloacae, Biofilm formation.

*Corresponding author at: Department of Biology, College of Science, University of Sulaimani, Kurdistan Region, Iraq.

E-mail address: hastyar.najmuldeen@univsul.edu.iq

Abstract

Aims: This study was designed to determine the extent of contamination of water storage tanks by non-lactose fermenter Enterobacter spp, and to characterize the chlorine and antibiotic resistance status. Finally, to find the correlation between biofilm formation and resistance to chlorine.

Methods: A total of 60 water samples were collected from residential and restaurant water storage tanks. Bacterial analysis and antibiotic susceptibility profiles of the samples were assessed by the most probable number (MPN) and Vitek 2 compact tests, respectively. The biofilm formation was quantified by crystal violet staining method and chlorine resistance test by microdilution technique.

Results: More coliform group contamination was recorded in residential- 40% than the restaurant water tanks 30%. Further evaluation of the samples assessed to be negative by the MPN test showed that 44% of home- and 43% of restaurant water samples were positive for Enterobacter cloacae. Further analysis of these isolates revealed that they displayed variation in resistance to different concentrations of chlorine, and similar antibiotic susceptibility profiles. Biofilm analysis showed no difference in biofilm formation, except the isolates that were resistant to concentration of chlorine, 400 mg L-1, formed significantly more biofilm than those that were resistant to other concentrations. A moderate positive non-linear correlation ($r = 0.72$) was found between the degree of biofilm formation and the ability of isolates to resist different chlorine concentrations ($p<0.05$), and no correlation has been detected between antibiotic and chlorine resistance.

Conclusions: The presence of E. cloacae in drinking water suggests a public health concern. The routine microbial water analysis should be modified to include detection of non-lactose fermenter Enterobacter.

Impact of Study: The presence of chlorine resistant, non-lactose fermenter Enterobacter spp in drinking water can pose a real public health threat. Therefore, the water samples should be routinely tested for the presence of Enterobacter spp.

Introduction:

Drinking water is essential for life, but it is also an excellent medium for transmission of numerous waterborne infections such as cholera, typhoid fever, infectious hepatitis, amoebic and bacillary dysentery (Khan et al., 2016). Chlorination is the most commonly used treatment method for water safety (Farkas et al., 2014, Levy et al., 2014, Shrivastava et al., 2004, Sun et al., 2013). However, many different species of bacteria are known to develop resistance to chlorine (Destiani and Science, 2019, Kamal et al., 2019, Sanganyado and Gwenzi, 2019). The increased prevalence of bacterial contamination of water storage tanks was linked to the loss of residual disinfectant activity and prolonged storage time (Akuffo et al., 2013, Brick et al., 2004, Chalchisa et al., 2018). The danger of contaminated water is not only restricted to the emergence of chlorine resistant bacteria, but chlorine resistance can lead to cross resistance to commonly used antibiotics (Kampf, 2018, Destiani and Science, 2019, Sanganyado and Gwenzi, 2019). Furthermore, the water distribution systems are known to harbor biofilms even in the continuous presence of disinfectants (Zhu et al., 2014). It is now accepted that microbial biofilms in drinking water distribution networks and water storage tanks can lead to the deterioration of water quality and pose a substantial threat to public health (Bertelli et al., 2018, Bridier et al., 2011). Biofilms protect contaminating bacteria and act as a potential reservoir for waterborne infections (Farkas et al., 2014).

International water quality standards depend mainly on the detection of total and fecal coliform Escherichia coli in pre- and post-chlorination (Bertelli et al., 2018, Cabral, 2010). While chlorination effectively eliminates fecal coliform E. coli, and other microbial species including Enterobacter spp. and Citrobacter freundii have been reported to be detected post-chlorination, very likely these resistant microbes are harboured in biofilms on water pipes and tanks (Cabral, 2010). Alarmingly, some of these Enterobacter spp were found to be multidrug resistant (MDR), which can cause healthcare-associated infections (Kanamori et al., 2016). A study conducted in the USA between 1995-2002, found that Enterobacter spp. are the significant cause of nosocomial bloodstream infections (Cabral, 2010). Two of the well- known Enterobacter spp, E. aerogenes and E. cloacae, are considered clinically important (Davin-Regli and Pagès, 2015, Mezzatesta et al., 2012).

The provision of safe drinking water is one of the major challenges of the 21st century (Cabral, 2010). Due to the water shortage, in Kurdistan and most of Iraqi cities , people rely on water storage tanks that are fixed to the house tops, which are directly exposed to sunlight and provides suitable growth condition for bacteria. In Iraq, public health authorities assess the microbial content in water samples using the most probable number (MPN) method. This method relies on detection of lactose fermenter E. coli, and does not take other contaminating bacteria into consideration. Therefore, the present study aimed to assess the extent of contamination by lactose fermenter E. coli and Enterobacter cloacae in water storage tanks in both residential dwellings and restaurants in Sulaymaniyah city. Furthermore, the isolates of E. cloacae were characterised for chlorine and antibiotic resistance as well as for biofilm forming ability.

Materials and Methods:

Sample collection

A total of 60 water samples were collected from residential and restaurant water storage tanks between August and September 2018. The samples (200 ml) were labeled and transported to the laboratory for standard (pH, temperature and residual chlorine) and bacteriological analyses at the directorate of health prevention hospital, Sulaymaniyah province of Kurdistan region in Iraq.

Microbiological water analysis:

Microbial content analysis of water samples is based on detection of total coliform, fecal coliform Escherichia coli, enterococci and Clostridium perfringens (Farkas et al., 2014). The Most Probable Number (MPN) technique is the most frequently used method for microbial quality assessment of water. In this study a single set of 5-tube MPN method was performed as recommend by the World Health Organization (WHO). The decision on the presence of total coliform E. coli was made on the basis of three successive steps, consisting of a presumptive test, a confirmation test (detection of thermotolerant fecal coliform), and a completed test (detection of Escherichia coli). In the first step, five tubes of 10 ml double-strength MacConkey broth was inoculated with 10 ml of water sample and incubated for 24 hours at 37°C. After incubation the presence of acid and gas in any tube was designated as a positive presumptive result. From the number of tubes with the positive reaction, the most probable number (MPN) of bacteria present in 100 ml of original water sample was determined using the probability table provided by the WHO. For the second step a 0.1 ml sample from the positive tube from step 1 was transferred into a single-strength MacConkey broth tube, and incubated for 24 hours at 44°C. After incubation the growth was recorded as a positive (thermotolerant fecal coliform) confirmed test. The final completed test was performed by inoculating Eosin Methylene Blue (EMB) agar plate and peptone broth with the positive growth from the confirmation test. The samples were incubated in duplicate at 35°C and 42°C for 24 hours. The growth was considered positive when green metallic sheen colonies on EMB agar and red ring formation on top of broth medium after addition of Kovac's reagent were seen.

To differentiate lactose fermenter from non-lactose fermenter bacteria, all MPN negative samples were sub-cultured on MacConkey agar. Then, the well-isolated colonies of non-lactose fermenter bacteria were subjected to further identification using Vitek-2 compact chlorine resistance test using microdilution technique, and antibiotic sensitivity test (AST) using both Vitek and disk diffusion tests (Kirbuy method) (Bauer et al., 1966).

Antibiotic susceptibility test by using Vitek-2 compact and disc diffusion method (Kirby Bauer test):

A loopful of non-lactose fermenter bacteria were streaked on nutrient agar and the plates were incubated at 37°C for 24 h. Then, a sufficient number of colonies from nutrient agar was suspended in 3 ml of sterile distilled water in order to obtain a turbidity level of 0.5-0.6 in McFarland scale. This bacterial suspension was used to determine the minimum inhibitory concentrations (MICs) and resistance patterns by the Vitek-2 Compact automated system using the AST-GN69 cards for the following antibiotics: Ampicillin, Amoxicillin/clavulanic acid, Cefazolin, Ceftazidime, Ciftriaxone, Cefepime, Entrapenem, Imipenem, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, and trimethoprim /sulfamethoxazole.

Furthermore, Kirby Bauer test was also used for determination of antibiotic sensitivity profiles as previously described (Bauer et al. 1966). Briefly, a sterile cotton swab was dipped into the microbial suspension and then was streaked evenly across the surface of a Muller-Hinton agar plate. Within 15 minutes of inoculation, the antimicrobial-containing disks (Amikacin, Vancomycin, Tetracycline, Metronidazol, and Chloramphenicol) were applied with a sterile forcep, and the plates were incubated at 37°C for 18 hours (Mahon and Manuselis, 2000). Results were interpreted using the M100-S25 of Clinical and Laboratory Standards Institute guidelines (CLSI-guideline 2015) (Patel et al., 2015).

***In vitro* determination of chlorine resistant bacteria**

Chlorine resistant bacteria were determined by microtiter plate assay according to procedure described by Huang et al. (2011) with slight modifications. Overnight cultures of bacterial strains were used for chlorine resistance test. Stock chlorine solutions were prepared according to the manufacturer's instructions (Chanelle Medical Limited Co, Ireland). Free chlorine residual in water was measured using previously described Mohr volumetric method (Mejia et al., 2016). From stock chlorine solution, six different chlorine concentrations of chlorine were prepared in sterile distilled water 25, 50, 100, 200, 300, and 400 mg L-1 and bacterial suspension separately prepared in sterilized distilled water (1×105 CFU mL-1). Then to mimic natural contact between chlorine concentrations and bacterial isolates, 2 µl of bacterial suspension thoroughly mixed with 200 µl (1:100 ratio) of each chlorine concentration using 96-microwell flat bottom polystyrene plates (SPL Plastic Labware, Korea). The plate was incubated at room temperature for 30 min as described previously (Destiani and Science, 2019, Yuan et al., 2015). After 30 min of contact time between bacteria and chlorine concentrations, the bacterial survival was measured by spotting 20 µl on Nutrient agar. Water containing no chlorine was used as a control.

Determination of biofilm formation:

The quantitative crystal violet staining method was used to quantify the level of biofilm formation as described previously (Suo et al., 2012, Tendolkar et al., 2004). Fresh nutrient broth medium was inoculated 1:100 with 1×105 CFU mL-1 of bacteria. Then, a 200 µl of this stock was transferred to the wells of 96-well flat bottom polystyrene plate (SPL Plastic Labware, Korea), and the plate was incubated in a static incubator at 37°C for 18 h. After removing planktonic cells, the wells were washed three times with 200 µl PBS. After drying at room temperature for 30 min, the biofilm was stained by addition of 200 µl 0.5 % (v/v) crystal violet at room temperature for 15 min. Excess crystal violet was removed by washing three times with PBS. Stained biofilm in each well was dissolved in 200 µl Ethanol : Acetone (80:20) (v/v). The absorbance of dissolved biofilm was measured at 595 nm using a microplate reader (Infinite F50 TECAN). The wells with uninoculated medium were used as control for crystal violet binding to the plastic.

Statistical Analysis

GraphPad prism version 6 (Graphpad, California, USA) was used to analyze all data. One-way ANOVA and Tukey's multiple comparisons tests were used for statistical analysis. p values less than 0.0001 was regarded statistically significant. Pearson correlation coefficient was used to analyze correlation between studied parameters, p value less than 0.05 was regarded statistically significant. Non-parametric Chi-square test was used to analyze dependence of positive result on the source of water samples, p value less than 0.1 was regarded statistically significant.

Results

Microbiological water analysis mainly focuses on detection of fecal indicator bacteria. The most common fecal indicator bacteria found in water are enterococci and coliform group, especially Escherichia coli (Cabral, 2010). In this study, the occurrence of total coliform, and fecal coliform bacteria in residential and restaurant water storage tanks were extensively studied. The results showed that the pH of samples ranged from 6.8 to 7.5, and the free chlorine from 0.01 to 1.12 mg L-1. The microbial analysis found more positive results (40%, 12/30) in residential than the restaurant water samples (30%, 9/30) (Figure-1A). Chi-square test showed that there is no significant dependence of positive result on the source of water samples (P>0.58), indicating that the probabilities of occurrence of fecal contamination in two sources of water storage tanks are similar. Moreover, more non-lactose fermenter Gram-negative bacteria are found in household water samples, 44% (8/18), than the restaurant samples, 43% (9/21) (Figure-1B), but the difference was statistically insignificant.

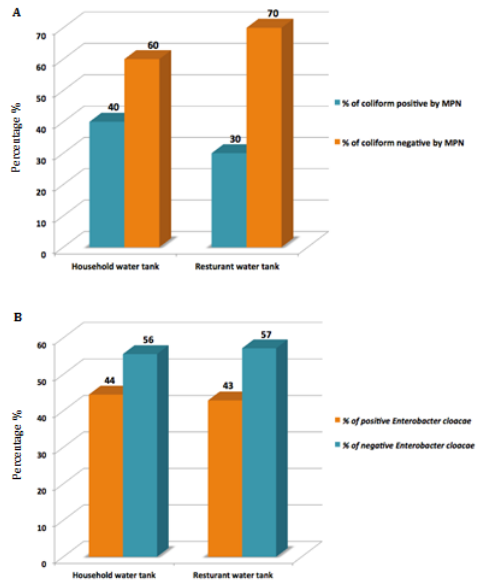

Figure 1: (A) Represents the coliform positive and negative water samples from home and restaurant storage tanks as assessed by the MPN method. (B) Shows the analysis of MPN negative samples for the presence of Enterobacter cloacae.

Determination of chlorine resistant non-lactose fermenter bacteria:

The present study has mainly focused on identification of Enterobacter cloacae and other related non-lactose fermenter pathogenic bacteria in water samples identified to be negative for coliforms using the MPN test. Results revealed Enterobacter cloacae as the only non-lactose fermenter species in the MPN negative water samples, found in 17 out of 39 analyzed samples. Then, the isolates were subjected to chlorine resistance test using different chlorine concentrations. The results showed that 5 isolates (G1) exhibited resistance to 25 mg L-1 chlorine, 3 isolates to 50 mg L-1 (G2), 4 isolates to 100 mg L-1 (G3), 2 isolates to 200 mg L-1 (G4), 1 isolate to 300 mg L-1 (G5), and 2 isolates (G6) were fully resistant to 400 mg L-1.

Biofilm formation:

The biofilm forming ability of E. cloacae strains was evaluated under static incubation using quantitative the crystal violet method. The results of correlation coefficient analysis exhibited moderate positive non-linear correlation (r = 0.722) between the degree of biofilm formation and the ability of isolates to resist different concentrations of chlorine ($p>0.05$). It was shown that G2 to G6 samples formed significantly more biofilm than the G1 samples ($p\leq0.0001$) (Figure-2). No significant differences in biofilm formation were noticed among G2 to G5 samples. The highest biofilm was produced by G6 samples, which were resistant to 400 mg L-1 of chlorine (Figure 2).

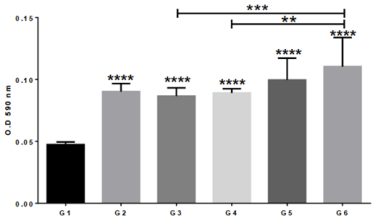

Figure-2: Evaluation of biofilm formation of E. cloacae strains by the crystal violet staining method. Data represent the mean ± SEM of three independent experiments; each with triplicates. One-way ANOVA and Dunnett's multiple comparison tests were used for statistical analysis (****$P\leq0.0001$). G1, G2, G3, G4, G5 and G6 strains were resistant to 25, 50, 100, 200, 300 and 400 mg L-1 chlorine, respectively.

Antibiotic susceptibility test by disc diffusion assay:

Residual chlorine was found to be the most important factor affecting the antibiotic resistance in bacteria in treated water samples. However, as a result of complexity of microbial community in natural environments the cross-resistance to chlorine and antibiotics have not been fully understood (Jia et al., 2015). Therefore, the occurrence of multidrug resistant (MDR) bacteria in water storage tanks has been investigated and correlation between chlorine and antibiotic cross-resistance was studied.

All isolates tested for chlorine resistance (G1 to G6) were analysed for resistance to 20 different antibiotics using Vitek compact-2 and a disc diffusion method recommended by CLSI-guidelines. The results revealed that all chlorine resistant isolates were sensitive to triprthoprim/sulfamethoxazole, ceftazidime, ceftriaxone, cefepime, ertapenem, imipenem, gentamicin, tobramycin, ciprofloxacin, levofloxacin, and chloramphenicol, but were resistant to rifampin, erythromycin, vancomycin, tetracycline, metronidazol, amoxicillin / clavulanic acid, cefazolin. A variable pattern of resistance and sensitivity were noticed for amikacin and nitrofurantoin as shown in (Table-1).

Table-1: Pattern of antibiotic resistance profiles of isolated Enterobacter cloacae strains resistance to different concentrations of chlorine; G1: included: W1-W5, G2: W6-W8, G3: W9-W12, G4: W13-W14, G5: W15, G6: W16-W17, 'W' referes to the water sample) was designated to different isolates of chlorine resistance E. cloacae. (S: Sensitive, R: Resistant, I: Intermediate)

Antimicrobial	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	W14	W15	W16	W17
Chlorine resistance (ppm)	25	25	25	25	25	50	50	50	100	100	100	100	200	200	300	400	400
Triprthoprim/Sulfamethoxazole	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Ceftazidime	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Cefepime	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Ertapenem	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Ceftazidime	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Imipenem	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Gentamicin	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Tobramycin	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Ciprofloxacin	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Levofloxacin	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Chloramphenicol	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
Rifampin	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)
Erythromycin	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)
Vancomycin	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)
Tetracycline	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)
Metronidazol	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)
Amoxicillin / Clavulanic acid	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)
Cefazolin	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)
Amikacin	(R)	(S)	(S)	(R)	(R)	(S)	(R)	(R)	(S)	(S)	(R)	(R)	(S)	(R)	(R)	(S)	(S)
Nitrofurantoin	(S)	(I)	(S)	(S)	(S)	(S)	(S)	(S)	(I)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)

Discussion:

According the WHO, the mortality rate due to water-associated diseases exceeds 5 million people every year (Cabral, 2010).

In Iraq, there are various factors that limit the chemical and microbial quality of potable public water including inadequate or non-existent sewage treatment, industrial waste contamination, and discharge of untreated hazardous chemicals. The report published by the WHO revealed that the water supply in Iraq is not enough to prevent a widespread humanitarian crisis in future (WHO, 2014). To remedy the water shortage, the majority of people rely on roof top water storage tanks. A study found that post-treatment water contamination by coliform bacteria and opportunistic pathogens is wide spread in these storage tanks (Tokajian and Journal, 2004).

The microbial analysis of the water samples from the storage tanks of residential dwellings and restaurants showed that 35% (21/60) were positive for fecal contamination, hence unsafe for human consumption. The problem of contamination in water storage tanks is common in many countries including in Dubai, (Khan et al., 2016) and in Bermuda, where nearly 45% and 66% (Tokajian and Journal, 2004), respectively, of analysed samples were found to be contaminated by E. coli and other bacterial species.

Chlorination is the main water treatment method in Kurdistan. However, chlorination does not guarantee the safety of drinking water, because water can get fecal contamination through leakage in the network. According to a previous study conducted in Sulaymaniyah city, while microbial content analysis of municipal storage tank did not show the presence of any contaminating bacteria, in the network and household samples, microbial growth could be demonstrated (Salih et al., 2015).

The microbial water quality test in Kurdistan and Iraq relies on the detection of fecal coliform E. coli. However, this test does not reveal the incidence of contamination by non-lactose fermenter E. cloacae which were found to be present in 43.6% of fecal coliform negative samples in this study, suggesting that chlorine resistance and biofilm former bacteria are prevalent in water storage tanks in Sulaimaniyah city. Previous studies emphasized that temperature, biofilm formation, and air greatly contribute in regrowth of different types of bacteria (Al-Bahry et al., 2013, Khan et al., 2016, Medrano Félix et al., 2011).

The frequent and overuse of cheap and non-toxic disinfectants like sodium hypochlorite (NaOCL) help in the development of chlorine resistance in bacteria (Kamal et al., 2019, Shrivastava et al., 2004).

Cabral (2010) documented that chlorine rapidly inactivates coliforms but leaves chlorine resistant pathogens unaffected. A previous study documented that Enterobacter spp. are the most frequently isolated organisms from tertiary treatment of wastewater (Leong et al., 1982).

The results of present study also showed that E. cloacae is able to tolerate up to 400 mg L-1 residual chlorine after 30 min contact time. In parallel to this finding Al-Berfkani et al. (2014) reported that Aeromonas hydrophilia, Staphylococcus aureus and Micrococcus varians resist 50, 100, 100 mg L-1 chlorine concentrations, respectively. However, other studies reported that 10 mg L-1 of free chlorine is enough to kill all bacteria except Gram-positives and spore-forming bacteria (Ridgway and Olson, 1982, Simões et al., 2010). These regional differences in cholorine resistance may be linked to differences in environmental conditions and local treatment regimens. The findings of this study demonstrated that E. cloacae isolates from water storage tanks in Sulaymaniyah are super resistance to chlorine.

The association between increased resistance to chlorine and biofilm formation ability of E. cloacae is consistent with the results of Bertelli et al. (2018) who emphasized that resistance of Pseudomonas to free chlorine is associated with their ability to form biofilm. Cell attachment is the first step of biofilm formation (Schroll et al., 2010), which is directly linked to exo-polysaccharide (EPS) production (Landini, 2009).

It has been found that production of exo-polysaccharide slime layer and biofilm formation restrict the diffusion of disinfectants into the inner layer of biofilm and may contribute to survival of P. aeruginosa in chlorinated water (Bertelli et al., 2018, Bridier et al., 2011, Grobe et al., 2001, Lin et al., 2017, Penna et al., 2002).

Additionally, the temperature, pH, and pipe and storage tank materials strongly encourage the process of biofilm formation, and chlorine-resistant community of Enterobacter spp. (Cabral, 2010, Zhu et al., 2014).

The results of this study revealed that the chlorine resistant E. cloacae are fully resistant to rifampin, erythromycin, vancomycin, tetracycline, metronidazole, amoxicillin / clavulanic acid, cefazolin, and amikacin, but no correlation was found between resistance to different chlorine concentrations and antibiotic resistance profiles. While several studies have reported the occurrence of many antibiotic resistant bacteria in sewage, treated drinking water and river water (Huang et al., 2012, Korzeniewska et al., 2013, Yuan et al., 2015), so far no clear physiological relation was found between mode of antibiotic action and resistance to different concentrations of residual chlorine. A previous study suggested that chlorination helps development of antibiotic resistance strains through transfer of resistance plasmids (Murray et al., 1984).

This concept has been confirmed by many researchers (Huang et al., 2011, Templeton et al., 2009). Similarly, a local study in the Khabur River, the main drinking water resource for Zahko-Duhok city in Iraq, showed that chlorination enhanced the antibiotic resistance in Staphylococcus aureus, Micrococcus varians and Aeromonas hydrophilia (Al-Berfkani et al., 2014).

However, other researchers found that chlorination did not significantly contribute to the resistance profile of E. coli strains (Iwane et al., 2001, Rizzo et al., 2013).

The lack of solid data on effectiveness of chlorination in reduction of antibiotic resistant bacteria requires further study to establish a link between antibiotic and chlorine resistance.

It can be concluded that water storage tanks in Sulaymaniyah city is contaminated with fecal E. coli and chlorine resistant E. cloacae. A positive moderate correlation was found between degree of chlorine resistance and biofilm formation. However, no correlation was noted between chlorine and antibiotic resistance among the E. cloacae strains. To control the potential risk posed by the waterborne pathogens, more effort should be dedicated to reduce the false sense of safety by widening the repertoire of tested indicator bacteria in microbial analysis of water samples. Moreover, the mechanism of chlorine resistance and its association with biofilm formation deserve to be studied in more detail in future.

Acknowledgment

The author would like to thank Sulaymaniyah water directorate for providing laboratory facilities, and to University of Sulaymaniyah and Komar University of Science and Technology, for providing continuous support during this work.

Conflict of Interest

The author has no conflict of interest to declare.

References:

AKUFFO, I., COBBINA, S. J., ALHASSAN, E. H. & NKOOM, M. 2013. Assessment of the quality of water before and after storage in the Nyankpala community of the Tolon-Kumbungu District, Ghana. International Journal of Science & Technology Research. 2(2):10-13.

AL-BAHRY, S. N., AL-HINAI, J. A. & PROCEDIA, M.-I. Y. 2013. Opportunistic and microbial pathogens in municipal water distribution systems. APCBEE procedia. 5: 339-343.

AL-BERFKANI, M. I., ZUBAIR, A. I. & BAYAZED, H. 2014. Assessment of chlorine resistant bacteria and their susceptibility to antibiotic from water distribution system in Duhok province. Journal of Applied Biology.2(6):10-13.

BAUER, A. W., KIRBY, W. M., SHERRIS, J.C., TURCK, M. 1966. Antibiotic Susceptibility Testing by a Standardized Single Disk Method, American Journal of Clinical Pathology. 45 (4): 493-496.

BERTELLI, C., COURTOIS, S., ROSIKIEWICZ, M. & et al. 2018. Reduced chlorine in drinking water distribution systems impacts bacterial biodiversity in biofilms. Frontiers in Microbiology. 9:1-11.

BRICK, T., PRIMROSE, B., CHANDRASEKHAR, R., ROY, S., MULIYIL, J., & KANG, G. 2004. Water contamination in urban south India: household storage practices and their implications for water safety and enteric infections. Int J Hyg Environ Health.5: 473-480.

BRIDIER, A., BRIANDET, R., THOMAS, V. & DUBOIS-BIOFOULING, F. 2011. Resistance of bacterial biofilms to disinfectants: a review. Biofouling. 27(9):1017-1032.

CABRAL, J. P. S. 2010. Water microbiology. Bacterial pathogens and water. Int. J. Environ. Res. Public Health. 7: 3657-3703.

CHALCHISA, D. MEGERSA, M. & BEYENE, A. 2018. Assessment of the quality of drinking water in storage tanks and its implication on the safety of urban water supply in developing countries. Environ Syst Res. 6(12):1-6.

DAVIN-REGLI, A. & PAGÈS, J.-M. 2015. Enterobacter aerogenes and Enterobacter cloacae; versatile bacterial pathogens confronting antibiotic treatment. Frontiers in Microbiology. 6:1-10.

DESTIANI, R. & SCIENCE, T.-M. R. 2019. Chlorination and ultraviolet disinfection of antibiotic-resistant bacteria and antibiotic resistance genes in drinking water. AIMS Environmental Science. 6(3):222-241.

FARKAS, A., BOCO B., BULARDA, M. D. & CRACIUNAS, C. 2014. Effect of different disinfectants against biofilm bacteria. Studia Universitatis Babes-Bolyai Biologia,LIX. 1: 5-20.

GROBE, S., WINGENDER, J. & FLEMMING, H-C. 2001. Capability of mucoid Pseudomonas aeruginosa to survive in chlorinated water. International journal of hygiene and Environmental Health. 204(2-3): 139-142.

HUANG, J. J., HU, H., LU, S. Q., LI, Y., TANG, F. & WEI, B. 2012. Monitoring and evaluation of antibiotic-resistant bacteria at a municipal wastewater treatment plant in China. Environ Int. 42:31-36.

HUANG, J. J., HU, H. Y., TANG, F., LI, Y., LU, S. Q., & LU, Y. 2011. Inactivation and reactivation of antibiotic-resistant bacteria by chlorination in secondary effluents of a municipal wastewater treatment plant. Water research. 45(9): 2775-2781.

IWANE, T., URASE, T. & YAMAMOTO, K. 2001. Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water. Water Science and Technology. 43(2): 91-99.

JIA, S., SHI, P., HU, Q., LI, B., ZHANG, T. & ZHANG, X. X.. 2015. Bacterial community shift drives antibiotic resistance promotion during drinking water chlorination. Environ Sci. Trchnol. 49(20): 12271-12279.

KAMPF, G. 2018. Biocidal agents used for disinfection can enhance antibiotic resistance in gram-negative species. Antibiotics. 7(4):1-24.

KAMAL, M. A., KHALAF, M. A., AHMED, Z. & JAKEE, J. E. 2019. Evaluation of the efficacy of commonly used disinfectants against isolated chlorine-resistant strains from drinking water used in Egyptian cattle farms. Vet. World. 12(12): 2025-2035.

KANAMORI, H., WEBER, D. J. & RUTALA, W. A. 2016. Healthcare Outbreaks Associated With a Water Reservoir and Infection Prevention Strategies. Clinical Infectious Diseases. 62(11):1423-1435.

KHAN, M. A., & ALMADANI, A. M. A. 2016. Assessment of microbial quality in household water tanks in Dubai, United Arab Emirates. Environ. Eng. Res. 22(1): 55-60.

KORZENIEWSKA, E., KORZENIEWSKA, A. & HARNISZ, M. 2013. Antibiotic resistant Escherichia coli in hospital and municipal sewage and their emission to the environment. Ecotoxicol. Environ. Saf. 91: 96-102.

LANDINI, P. 2009. Cross-talk mechanisms in biofilm formation and responses to environmental and physiological stress in Escherichia coli. Research in microbiology.160(4): 259-266.

LEONG, L. Y. C., OTSAKA, D. & RIDGWAY, H. F. 1982. Chlorine-Resistance of Coliform-Tested Bacteria Isolated from Raw and Treated Sewage Effluents. Water Sci Tecnol. 14(4-5): 127-132.

LÉVESQUE, B., PEREG, D., WATKINSON, E., MAGUIRE, J. S., BISSONNETTE, L., GINGRAS, S., ROUJA, P., BERGERON, M. G. & DEWAILLY, É. 2008. Assessment of microbiological quality of drinking water from household tanks in Bermuda. Can J Microbiol. 54(6): 495-500.

LEVY, K., ANDERSON, L., ROBB, K. A., CEVALLOS, W., TRUEBA, G. & EISENBERG J. N. 2014. Household effectiveness vs. laboratory efficacy of point-of-use chlorination. Water Res. 54:69-77.

LIN, H., ZHU, X., WANG, Y., YU, X. 2017. Effect of sodium hypochlorite on typical biofilms formed in drinking water distribution systems. Journal of water and health. 218-227.

LOCKHART, S. R., ABRAMSON, M. A., BEEKMANN, S. E., GALLAGHER, G., RIEDEL, S., DIEKEMA, D. J., QUINN, J. P. & DOERN, G. V. 2007. Antimicrobial Resistance among Gram-Negative Bacilli Causing Infections in Intensive Care Unit Patients in the United States between 1993 and 2004 J Clin. Microbiol. 45(10): 3352-3359.

MAO, G., SONG, Y., BARTLAM, M. & WANG, Y. 2018. Long-term effects of residual chlorine on Pseudomonas aeruginosa in simulated drinking water fed with low AOC medium. Front Microbiol. 9: 1-10.

MEDRANO FÉLIX, A., MARTINEZ, C., CASTRO-DEL, C.N, LEON-FELIX, J, PERAZA-GARAY, F. GERBA, C. P. & CHAIDEZ, C. 2011. Impact of prescribed cleaning and disinfectant use on microbial contamination in the home. J Appl. Microbiol. 110(2): 463-470.

MEZZATESTA, M. L., GONA, F. & STEFANI, S. 2012. Enterobacter cloacae complex: clinical impact and emerging antibiotic resistance. Future microbiol. 7(7): 887-902.

MEJIA, J., Michałowska-Kaczmarczyk, A. & Michałowski, T. (2016). Mohr's method challenge. Anal Bioanal Chem . 408:1721–1722.

MILES, A. A., MISRA, S. S. & IRWIN, J. O. 1938. The estimation of the bactericidal power of the blood. J Hyg. 38(6): 732-749.

MURRAY, G. E., TOBIN, R. S., JUNKINS, B., & KUSHNER, D. J. 1984. Effect of chlorination on antibiotic resistance profiles of sewage-related bacteria. Applied and Environmental Microbiology. 48(1): 73-77.

PENNA, V., MARTINS, S. & MAZZOLA, P. 2002. Identification of bacteria in drinking and purified water during the monitoring of a typical water purification system. BMC Public Health. 2(13):1-11.

RIDGWAY, H. F. & OLSON, B. H. 1982. Chlorine resistance patterns of bacteria from two drinking water distribution systems. Appl. Environ. Microbiol. 44(4): 972-987.

RIZZO, L., MANAIA, C., MERLIN, C., SCHWARTZ, T., DAGOT, C., PLOY, M. C., MICHAEL, I. & FATTA-KASSINOS, D. 2013. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. Sci Total Environ. 447: 345-360.

SANGANYADO, E. & GWENZI, W. 2019. Antibiotic resistance in drinking

water systems: Occurrence, removal, and human health risks. Science of The Total Environment. 669: 785-797.

SALIH, F. A., OTHMAN, N., MUHIDIN, F. M. and KASEM, A. O. 2015. Assessment of the Quality of Water inSulaimaniyah City, Kurdistan Region: Iraq. Current World Environment. 10(3): 781-791.

SCHROLL, C., BARKEN, K. B., KROGFELT, K. A. & STRUVE, C. 2010. Role of type 1 and type 3 fimbriae in Klebsiella pneumoniae biofilm formation. BMC Microbiol.10: 179.

SHRIVASTAVA, R., UPRETI, R. K., JAIN, S. R., ORASAD K. N., SETH, P. K. & CHATURVEDI, U. C. 2004. Suboptimal chlorine treatment of drinking water leads to selection of multidrug-resistant Pseudomonas aeruginosa. Ecotoxicol. Environ. Saf. 58(2): 277-283.

SIMÕES, L., SIMÕES, M. & VIEIRA, M. 2010. Influence of the Diversity of Bacterial Isolates from Drinking Water on Resistance of Biofilms to Disinfection ⊠ Applied and Environmental Microbiology. 76(19): 6673-6679.

SUN, W., LIU, W., CUI, L., ZHANG, M. & WANG, B. 2013. Characterization and identification of a chlorine-resistant bacterium, Sphingomonas TS001, from a model drinking water distribution system. Sci. Total Environ. 458-460:169-175.

SUO, Y., HUANG, Y., LIU, Y., SHI, C. & SHI, X. 2012. The expression of superoxide dismutase (SOD) and a putative ABC transporter permease is inversely correlated during biofilm formation in Listeria monocytogenes 4b G. PLoS One. 7(10):1-9.

TEMPLETON, M. R., ODDY, F., LEUNG, W-K. & ROGERS, M. 2009. Chlorine and UV disinfection of ampicillin-resistant and trimethoprim-resistant Escherichia coli. Canadian Journal of Civil Engineering. 36(5): 889-894.

TENDOLKAR, P. M., BAGHDAYAN, A. S., GILMORE, M. S. & SHANKAR, N. 2004. Enterococcal Surface Protein, Esp, Enhances Biofilm Formation by Enterococcus faecalis. Infect. Immun, 72 (10): 6032-6039.

TOKAJIAN, S. & HASHWA, F. 2004. Microbiological quality and genotypic speciation of heterotrophic bacteria isolated from potable water stored in household tanks. Water Quality Research Journal. 39(1): 64-73.

YUAN, Q. B., GUO, M. T. & YANG, J. 2015. Fate of antibiotic resistant bacteria and genes during wastewater chlorination: implication for antibiotic resistance control. PloS one. 1-11.

ZHU, Z., WU, C., ZHONG, D., YUAN, Y., SHAN, L. & ZHANG, J. 2014. Effects of pipe materials on chlorine-resistant biofilm formation under long-term high chlorine level. Applied biochem Biotechnol.173 (6): 1-16.

World Health Organization WHO (2014). Conflict and humanitarian crisis in Iraq, public health risk assessment and intervention