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USING DISSEMINATION OF ANTIBIOTIC RESISTANT BACTERIA (ARB) AND RESISTANCE GENES (ARG) FOR WASTEWATER TREATMENT

MD.Zunaud Siddiki Mamoon[1],Omar Al Maimun[2]
Lecturer, Civil Engineering, Rangpur Engineering Institute, Bangladesh
Researcher, Hydrology and Water Resources, Hohai University, Nanjing, China.

ABSTRACT: *This investigation was directed to assess the impact of wastewater treatment forms on the pervasiveness of anti-infection obstruction fecal coliform (FC) and anti-toxin opposition qualities (ARGs) of FC. What's more, the event of anti-infection safe microscopic organisms (ARB) and anti-toxin safe qualities (ARGs) in surface waters accepting wastewater was assessed. More noteworthy opposition against penicillin (P), colistin (CT) and ampicillin (AMP) were watched for FC disconnected from profluent purified by chlorine (71%), than that cleaned by UV (45%). The best opposition against six anti-infection agents was recorded for FC secludes from emanating purified by chlorine. The pervasiveness of test and blaSHV was most reduced in disconnects from chlorine-purified effluents. The event of ARG blaSHV was most astounding in FC disconnected from emanating sterilized by UV. A critical relationship was recorded between FC levels in surface waters and the degree of bacterial protection from ampicillin ($P < 0.05$) and to chloramphenicol ($P < 0.05$). AmpC and blaPSE1 were more pervasive than blaSHV in effluents and in surface waters. TetA and tests were profoundly pervasive in surface water contrasted with test. The consequences of the examination exhibit across the board pervasiveness of ARB and ARG in wastewater and accepting water bodies. The outcome shows that the wellspring of ARB and ARG in surface waters start from wastewater. Discharged ARB and ARG may fill in as the wellspring of ARG to pathogenic microscopic organisms in surface waters. Sanitization procedures may impact the choice of anti-infection safe examples of microorganisms.*

KEYWORDS: Wastewater, Antibiotic Resistant Bacteria, Genes, Treatment, Disinfection

INTRODUCTION

The widespread application of antibiotics in human and veterinary medicine has led to the emergence, selection, and dissemination of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in different environmental compartments throughout the world. Currently, there is a lack of knowledge with respect to the origin of ARB and ARGs in different surface waters (e.g. stream water and bathing water) and their removal by advanced wastewater treatment processes. Antibiotic resistance gained increased attention in the past years—not least due to alarming reports of the World Health Organization (WHO). Studies demonstrated the presence of antibiotic resistant bacteria in clinical, domestic and industrial wastewaters [1] [2] [3]. The resistant bacteria reach wastewater treatment plants, where currently insufficient microbial reduction is accom-plished. Therefore,

antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) were found in surface water, groundwater, drinking water and se-diments in various regions of the world [4] [5] [6] [7]. The dissemination of ARB and ARGs is facilitated by the horizontal gene transfer enabling the ex-change of ARGs among different strains or bacterial species [8] and beyond the habitat of the original host [9]. Wastewater treatment plants (WWTPs), espe-cially activated sludge, provide an opportunity for mobile genetic elements (in-cluding ARG) to mix between pathogens, opportunistic pathogens, and envi-ronmental bacteria [10]. The effect of clinically relevant ARGs and ARB, that are released, from anthropogenic sources, together with the excessive use of antibio-tics in both human and veterinary medicine, is currently considered a serious public health issue. It is assumed that the global spread of ARGs and ARB and the acquisition of the resistance genes by pathogenic bacteria are associated with the increased hospitalization and mortality rates of patients that are infected with such organisms [11]. The treatment of infectious diseases becomes more difficult and the number of deaths associated with antibiotic resistant bacteria increases worldwide. Antibiotic resistance is commonly associated with extra chromosomal elements, which include different types of mobile DNA segments such as plasmids, transposons and integrons. The introduction of mobile DNA elements is accomplished through the processes of transduction, conjugation and transformation. It is important to note that gene transfer can occur in the same species and in genetically distant species such as gram-positive and gram-negative bacteria. Among the mechanisms, which were developed by bateria for multidrug resistance (MDR) are: penicillin-binding proteins (PBPs), enzymatic mechanisms of drug modification, mutated drug targets, enhanced ef-flux pump expression and altered membrane permeability [12]. This study was performed to evaluate the influence of wastewater treatment processes on the prevalence of antibiotic resistance fecal coliform (FC) and antibiotic resistance genes of FC. Furthermore, the influence of wastewater treated effluents on the dissemination of ARB and ARG in receiving surface waters was evaluated. The Yarkon Stream was selected for this study because it receives treated wastewater effluents and it flows into the Mediterranean Sea, as a result the ARB and ARG carried by the stream may be the source of ARG to pathogenic bacteria in the marine environment.

MATERIALS AND METHODS

Samples Collection

Samples of secondary and tertiary wastewater effluents (disinfected by either chlorine or UV) were collected from two wastewater treatment plants located in the central part of Israel. The treatment train consists of primary settling, acti-vated sludge and tertiary treatment (filtration and disinfection by either chlorine or UV irradiation). One-liter grab samples were collected, held at 4˚C during transportation to the laboratory and assayed within 2 hrs, for the prevalence of AR FC and ARG of

FC. To determine the influence of wastewater effluent dis-charged into surface water on the transmission of ARB and ARG, the following samples were analyzed: 1) secondary, 2) tertiary effluents disinfected by UV, 3) irrigation reservoir receiving tertiary effluents, 4) Yarkon stream, 5) Yarkon outfall into the Mediterranean Sea, and 6) low impact environment (Mediterra-nean Sea). Forty two samples were collected and transferred to the laboratory. The same samples were used for either ARB or ARG analysis.

Fecal Coliform Isolation

Detection and enumeration of fecal coliform was performed following the procedure of Standard Methods [13]. Enumeration of FC in samples of tertiary ef-fluent, irrigation reservoir and low impact seawater was accomplished by the membrane filtration. On the other hand, samples of secondary effluent, stream water and stream outfall were diluted for the isolation of fecal coliform.

Antibiotic Resistance of Fecal Coliform Isolates

The antimicrobial susceptibility of fecal coliform isolates was determined by the disc-diffusion method (Oxoid, Ballerup, Denmark). Disks containing the fol-lowing antimicrobial agents were used as representatives of important antibiotic classes: Ampicillin (AMP, 10 µg), Chloramphenicol (C, 30 µg), Gentamicin (CN, 10 µg), Cephalotin (KF, 5 µg), Kanamycin (30 µg), Nitrofurantoin (F, 300 mg), Amoxicillin (AMC, 30 µg), Ciprofloxacin (CIP, 5 µg), Colisitin (CT, 25 µg), Penicillin (10 unit), Streptomycin (S, 10 µg) and *tetracycline* (TE, 25 µg). Fecal coliform isolates were inoculated onto Mueller-Hinton agar (Merck) plates. The various antibiotic disks were placed on the inoculated Mueller-Hinton agar plates. After overnight incubation at 37˚C, inhibition zones around each disk were measured to the nearest millimeter. The results were analyzed using the criteria of the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS) [14]. The standard strain *Escherichia coli* K12 was used for quality control [15].

Enumeration of Antibiotic Resistant Heterotrophic Bacteria

Antibiotic resistant heterotrophic bacteria in effluents and surface water samples were determined by heterotrophic plate count (HPC). Samples from secondary effluents and stream outfall were diluted, while samples of tertiary effluents, re-servoir, stream and seawater were filtered through a 0.22 µM-pore membrane (Millipore, Billerica, MA) and then placed on Mueller Hinton agar containing the following antibiotics, *tetracycline* (20 ug/ml), ampicillin (60 ug/ml) or chlo-ramphenicol (10 ug/ml). Plates were incubated along with the negative and posi-tive controls at 37˚C for 24 h.

DNA Extraction

Genomic DNA was extracted directly from 25 fecal coliform colonies isolated from secondary or tertiary effluents disinfected by either chlorine or UV. DNA of these multidrug resistant isolates was extracted using QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to manufactures instructions. Extracted nucleic acid was stored at −20˚C prior to analysis.

To detect ARG from wastewater effluents and surface water samples, DNA was extracted from 50 mL of each sample by filtration onto a sterile 47-mm 0.22 µM polycarbonate filter (Millipore). Filters were placed into a Mo Bio PowerSoil (Mo Bio Laboratories Inc., Carlsbad, CA) tube and DNA was extracted from the filters following the manufacturer's protocol. The purity and quantity of the ex-tracted DNA were measured by a Nanodrop ND-1000 UV-visible light spectro-photometer (Nanodrop Technologies, Wilmington, DE). The extracted DNA was stored at −20˚C until assay.

PCR Detection of ARGs

Multi drug resistant fecal coliform isolates were divided into three groups sec-ondary effluent (n = 25), tertiary effluent disinfected by UV (n = 25) and tertiary effluent disinfected by chlorine (n = 25). The fecal coliform isolates were screened for the presence of five antibiotic resistance genes. ARGs include *β*-lactam resistance genes (ampC, bla_{SHV}) and *tetracycline* resistance genes (tetA, tetB). Previously published primer sets were used for the PCR amplification of ARGs [4].Polymerase Chain Reaction (PCR) was used for the detection of beta lactams (ampC, bla_{SHV}, blapse1), *tet*racycline (tetA, tetB, tetC) *and* chloramphenicol (CAT and Flor) genes in total DNA extracted from secondary effluent, tertiary effluent, reservoir, stream, stream outfall and seawater (low impact) samples (n=42). PCR was performed with (SimpliAmp Applied Biosystems, USA). A total 42 samples obtained from the six sampling sites (7 samples each) along with control DNA extracted from *E. coli* (ATCC 25922), previously, published pri-mers were used for the PCR amplification of ARGs [4]. The PCR conditions for ARGs amplification were similar to those reported by Stoll *et al.* (2012) [4]. Ten microliters of amplified product including positive and negative controls (sterile water) were electrophoresed on a 0.5% agarose gel containing GelRed stain. 100 bp DNA ladder (Biolabs, New England) was used as a standard DNA ladder.

Statistical Analysis

The chi-squared test was used to compare the prevalence of antibiotic resistance phenotypes and sampling site (include wastewater treatment process) among the fecal coliform isolates (against 12 antibiotics) and total heterotrophic antibiotic resistant bacteria (against ampicillin, *tetracycline* and chloramphenicol). Statis-tical analysis was performed using Microsoft Excel version 2007 for Windows. A *p*-value of <0.001 (by use of the Bonferroni correction) or <0.05, considered sta-tistically significant. Pearson correlation was conducted to identify the associa-tion between the concentrations of fecal coliform (indicator for fecal contamina-tion) and antibiotic resistant HTB against ampicillin, *tetracycline* and chloram-phenicol among the effluent and surface water samples.

RESULTS AND DISCUSSION

Occurrence of AR Fecal Coliform in Secondary Effluent and in Tertiary Effluent Disinfected by Chlorine or UV

Fecal coliform isolated from wastewater effluents were highly resistant (90% to 100%) to penicillin and colisitin (**Table 1**). Higher resistant to ampicillin was recorded for FC isolated from tertiary effluent disinfected by chlorine as com-pared 76% of FC isolated from tertiary effluent disinfected by chlorine were found to be resistant to ampicillin, compared to 42% and 44% of FC isolated from secondary effluent and tertiary effluent disinfected by UV. Resistance to streptomycin and *tetracycline* was similar for FC isolated from secondary efflu-ent (16% and 16%) and tertiary effluent disinfected by UV (29% and 38%), whe-reas lower level of resistance to streptomycin and *tetracycline* (7% and 8%) was recorded for FC isolated from tertiary effluent disinfected by chlorine. Wastewater treatment may enhance the selection of ARB especially, after ex-posure to disinfection agents such as chlorine. Although, chlorination of waste-water effluent may reduce the concentration of FC, it may substantially increase the proportions of ARB. This observation was recorded for FC resistant to AMP and AMC isolated from chlorinated effluent. However, similar AR patterns were observed for secondary effluent and UV disinfected effluent, which indicates that UV at the doses used for effluent disinfection does not result in further AR selection.

Prevalence of Multi Drug Resistant (MDR) FC in Secondary Effluent and in Tertiary Effluent Disinfected by Chlorine or UV

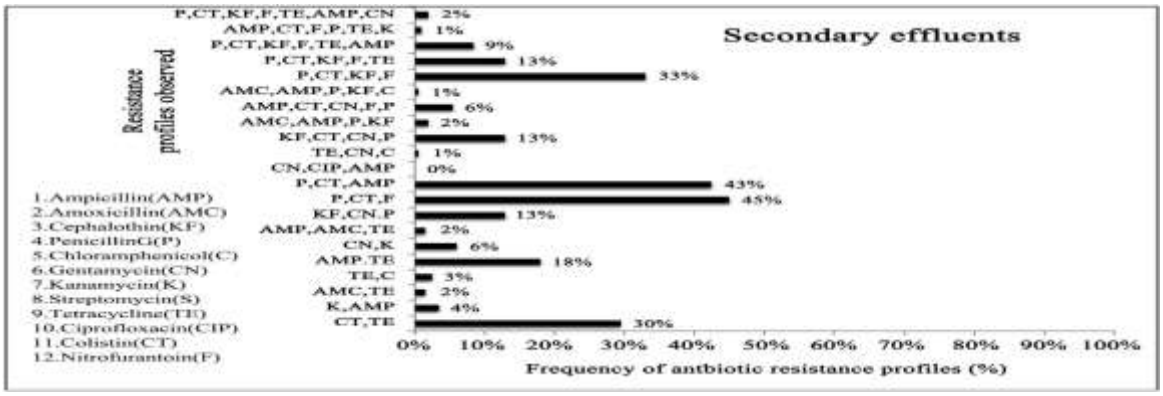
Results of the prevalence of MDR fecal coliform in secondary effluent, tertiary effluent disinfected by either chlorine or UV irradiation are presented in **Figure 1**. A fecal coliform isolate was considered MDR when it showed resistance to

Antibiotic	% fecal coliform AR in		
	Secondary Effluent (n = 200)	Tertiary effluent UV disinfection (n = 150)	Tertiary effluent Chlorine disinfection (n = 150)
Ampicillin (AMP, 10 µg)	42*	44	76
Chloramphenicol (C, 30 µg)	2	11.5	3
Gentamicin (CN, 10 µg)	17	18	12
Cephalotin (KF, 5 µg)	70	69	51
Kanamycin (K, 30 µg)	6	8	11
Nitrofurantoin (F, 300 mg)	44	63	44
Amoxicillin (AMC, 30 µg)	2	5	32
Ciprofloxacin (CIP, 5 µg)	8	9	5
Colisitin (CT, 25 µg)	97	98	90
Penicillin (P10 unit)	100	99	99
Streptomycin (S, 10 µg)	16	16	7
***Tetracycline* (TE, 25 µg)**	29	38	8

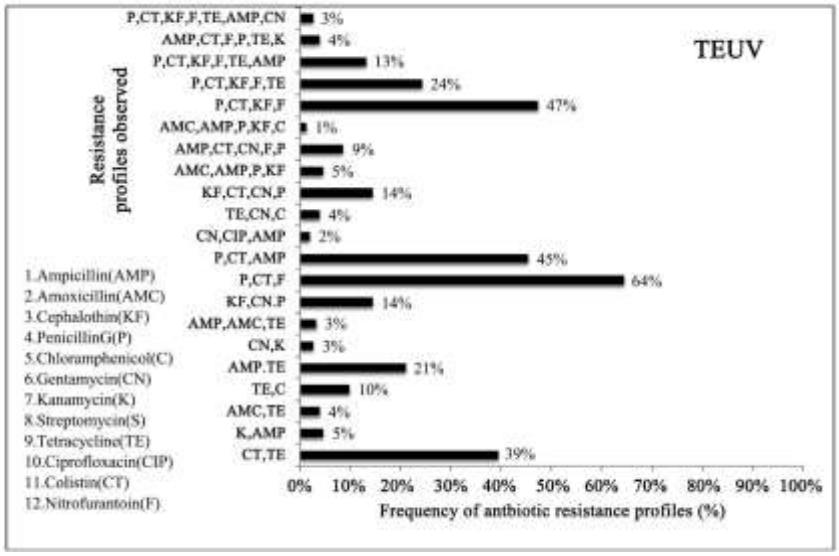
more than 3 antibiotics. The highest percentage of FC resistant to 3 antibiotics was recorded for P, CT and AMP, where 71% of the isolates from tertiary efflu-ent disinfected by chlorine were found resistant. In comparison, 43% and 45% of FC isolated from secondary effluent and tertiary effluent disinfected by UV were found resistant to the same three antibiotics, respectively.

Table 1. Resistance of fecal coliform isolates from secondary effluent (n = 200), from tertiary effluent disinfected by chlorine (n = 150) or by UV irradiation to 12 types of antibiotics

%fecal coliform AR was calculated as number of resistant colonies divided by total number of isolated co-lonies.



(a)



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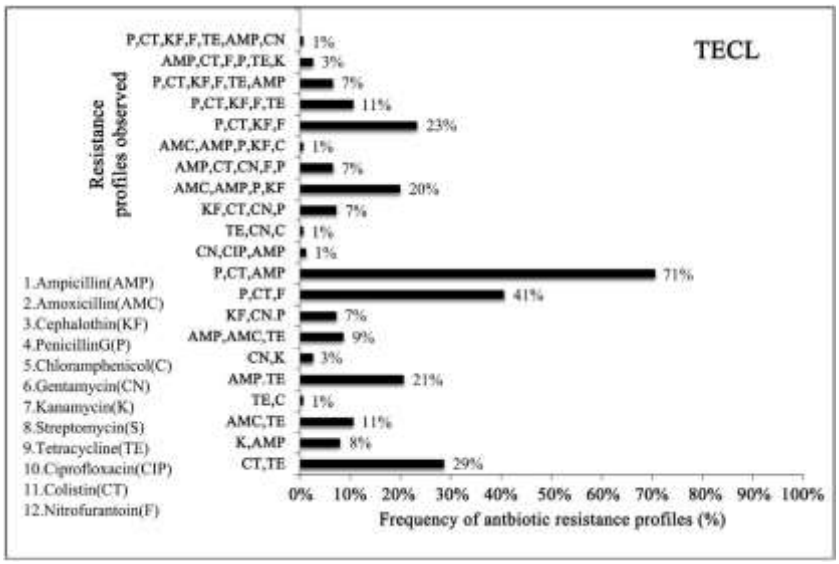

(c)

Figure 1. Profile of Multi drug resistant fecal coliform isolated from: (a) secondary effluent, (b) tertiary effluent disinfected by UV irradiation and (c) tertiary effluent disinfected by free chlorine.

The highest level (47%) of FC resistant to 4 antibiotics (P, CT, KF and F) was recorded for isolates from tertiary effluent disinfected by UV irradiation. In comparison, lower levels of FC MDR to 4 antibiotics were detected in secondary effluent (33%) and tertiary effluent disinfected by UV. The highest difference for MDR resistant to 4 antibiotics (AMC, AMP, P

and KF) was observed for FC iso-lated from tertiary effluent disinfected by chlorine (20%) in comparison to 5% of FC isolated from tertiary effluent disinfected by UV and 2% FC isolated from secondary effluent.For MDR Fecal coliform resistant to 5 antibiotics (P, CT, KF, F and TE) the highest level (24%) was recorded for isolates from tertiary effluent disinfected by

UV irradiation, as compared to 11% and 13% of FC isolates from tertiary efflu-ent disinfected by chlorine and secondary effluent, respectively. MDR for 6 anti-biotics (P, CT, KF, F, TE and AMP) was recorded for 13%, 9% and 7% of FC isolated from tertiary effluent disinfected by UV irradiation, secondary effluent and tertiary effluent disinfected by chlorine, respectively. It is worth noting that low percentage (up to 3%) of FC isolates were MDR to even 7 antibiotics (P, CT, KF, F, TE, AMP and CN). Although, most FC released to the environment either for water reuse in irrigation or discharged into surface water bodies are not pa-thogenic, the high level of MDR detected in the treated effluents may introduce a high risk of spreading AR to environmentally transmitted pathogenic bacteria. MDR fecal coliform may be the source of horizontal transfer of antibiotic resis-tant genes. Our results show that disinfection procedures can result in differenc-es in resistance patterns of FC to antibiotics. Huang *et al.* (2011) demonstrated that the inactivation of *tetracycline*-resistant *E. coli* was found significantly low-er than that of antibiotic-sensitive *E. coli* at high chlorine doses [16]. However, opposite results were observed for ampicillin- and trimethoprim-resistant *E. coli* [17]. These authors suggested that chlorination does not select for ampicillin-and trimethoprim-resistant *E. coli* through water treatment processes [17].

Occurrence of Antibiotic Resistant Genes (ARG) of MDR Fecal Coliform

Fecal coliform isolates found resistant to *tetracycline* and ampicillin using disk diffusion method were selected for the detection of genes associated with anti-microbial resistance using PCR. MDR fecal coliform isolates from secondary ef-fluent (n = 25) and tertiary effluent treated either by chlorine (n = 25) or UV (n+25) were screened for the presence of the following antimicrobial resistance genes: *tet*racycline (tetA and tetB) and *β*-lactamases (ampC and bla_{SHV}). The highest prevalence (100%) was observed for ampC gene in MDR isolated from secondary effluent and tertiary effluent disinfected by UV, whereas ampC gene was detected in only 50% of FC isolates from tertiary effluent disinfected by chlorine (**Table 2**). The gene bla_{SHV} was more frequently detected in MDR fecal coliform isolates from tertiary effluent

disinfected by UV (83%) than secondary and tertiary effluent disinfected by chlorine (28% and 10%), respectively. The MDR fecal coliform isolates were analyzed for the presence of two efflux pump encoding *tetracycline* resistance genes tet(A) and tet(B). The tet(A) gene was found to be more prevalent in tertiary effluent disinfected by UV (52%) than in MDR isolates from secondary effluents (44%) and MDR isolates from tertiary ef-fluent disinfected by chlorine (25%), In comparison, tet(B) was found more fre-quently in MDR fecal coliform isolates from secondary effluent (88%) and in MDR fecal coliform from tertiary effluents disinfected by UV (74%) than in MDR isolates from tertiary effluents disinfected by chlorine (5%) (**Table 2**). Pre-vious study by Munir *et al.*, 2001 found out that disinfection by chlorination and UV radiation did not significantly reduce ARGs and ARB and no difference was observed between the disinfection processes [18]. On the other hand, the appli-cation of sequential UV disinfection followed by chlorination significantly re-duced the ARGs and had synergistic effects compared to single disinfectant use [19]. Moreover, Öncü *et al.* (2011) found out that the sensitivity to the antibio-tics amoxicillin, ciprofloxacin and sulfamethoxazole was not altered in chlorine resistant *E. coli* [20]. Our results, although limited number of analyzed colonies, suggest that disinfection process may select for antibiotic resistant fecal coliform.

Prevalence of Antibiotic Resistant Heterotrophic Bacteria(ARHTB) in Wastewater Effluents and Receiving Surface Water

The prevalence of ARHTB in wastewater effluent and receiving surface waters was tested using ampicillin, *tetracycline* and chloramphenicol (**Figure 2**). The concentration of HTB resistant to ampicillin was the highest in the Yarkon out-fall samples (up to 6×10^5 cfu/100ml). The concentration of ampicillin resistant HTB in secondary effluent, tertiary effluent, wastewater irrigation reservoir and the Yarkon stream was lower than that recorded for the outfall, but was in the same order of magnitude. The concentration of ampicillin resistant HTB in the low impacted seawater was very low (90 cfu/100ml). The correlation between FC concentration and the level of HTB resistant to ampicillin was significant ($R = 0.93$, $P < 0.05$).

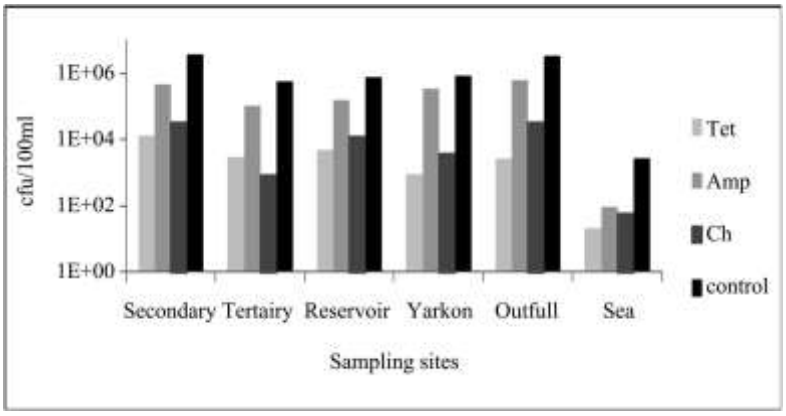

Figure 2. Concentration of heterotrophic bacteria resistant to ampicillin (A), *tetracycline* (B) and chloramphenicol (C) in wastewater effluents and receiving surface waters.

Table 2. Prevalence of antibiotic resistant genes (ARG) of MDR fecal coliform.

Antibiotic resistant gene	**Prevalence (%) of ARG in**		
	Secondary effluent (n = 50)	Tertiary effluent UV (n = 25)	Tertiary effluent Chlorine (n = 25)
tet A	44%	52%	25%
tet b	88%	74%	5%
ampC	100%	100%	50%
Bla SHV	28%	83%	10%

The highest concentration of *tetracycline* resistant HTB was observed in sec-ondary effluent samples (1×10^4 cfu/100ml), followed by samples from reservoir, tertiary effluent and Yarkon outfall. While the lowest concentration of HTB re-sistant to *tetracycline* in the receiving surface waters was recorded in the Yarkon stream (9×10^2 cfu/100ml). The low impacted seawater contained 20 cfu/100ml *tetracycline* resistant HTB. Weak correlation was recorded between FC concen-tration and the concentration of HTB resistant to *tetracycline* ($R = 0.37$, $P < 0.05$).Similar concentration of HTB resistant to chloramphenicol was observed in secondary effluent and stream outfall (10^4 cfu/100ml). Only 60 cfu/100ml HTB resistant to chloramphenicol were detected in the low impacted seawater. Signif-icant correlation was

recorded between FC concentration and the concentration of HTB resistant to chloramphenicol ($R = 0.95$, $P < 0.05$).

In general, the concentration of HTB resistant to ampicillin in the various water sources was highest followed by HTB resistant to chloramphenicol and lowest was recorded for *tetracycline*. The level of FC detected in the stream out-fall samples suggest that additional wastewater contamination may be dis-charged close to the sampling location.

Prevalence of ARG in Wastewater Effluents and Receiving Surface Waters

Samples of wastewater effluent and receiving surface waters were screened for the presence of 8 ARGs which included; *tetracycline* resistance genes (tetA, tetB, tetC), *β*-lactams resistance genes (ampC, bla_{SHV}, blapse1), and chloramphenicol resistance genes (Flor, and Cat).The most prevalent detected gene was *ampC*, which was detected in all sam-ples (100%), except in the low impacted seawater samples (29%) (**Table 3**). In comparison, 57% of samples from secondary, tertiary effluents and the stream outfall harbored bla_{SHV} gene, while bla_{SHV} gene was detected in only 29% of sam-ples from the irrigation reservoir and Yarkon stream the bla_{SHV} gene was de-tected. The lowest level of bla_{SHV} gene (14%) was detected in the low impacted seawater samples. The ARG *blapse*1 was highly prevalent in samples of effluents, reservoir, and Yarkon Stream (100%) and found at lower prevalence in low im-pacted seawater (43%).The prevalence of three efflux pump genes (tetA, tetB, and tetC) encodes re-sistance against *tetracycline* were examined in wastewater effluents and receiving water bodies (**Table 3**). Genes tetA and tetC were highly prevalent (100%) in all tested samples, whereas tetA was completely absent and tetC was detected in 29% samples from low impact sea samples. In comparison, tetB was prevalent in all secondary effluent samples (100%), followed by samples from the Yarkon outfall and the irrigation reservoir (57%). In tertiary effluents and outfall sam-ples tetB was present in only 29% of the samples. Gene tetB was detected in only 14% of the analyzed low impact seawater samples.

Table 3. Prevalence of ARG in wastewater effluents and receiving surface water.

Antibiotic resistant gene to	**gene**	**SW**	**TE**	**Res**	**Stream**	**Outfall**	**Low** impact
Tetracycline	*tet* A	100%	100 %	100%	100%	100%	0%
	tet B	100%	29%	57%	29%	57%	14%
	tet C	100%	100 %	100%	100%	100%	29%
β-lactams	ampC	100%	100 %	100%	100%	100%	86%
	bla_{SHV}	57%	57%	29%	29%	57%	14%
	blapsel	100%	100 %	100%	100%	86%	43%
chloramphenicol	Flor	100%	71%	100%	86%	100%	0%
	Cat	100%	100 %	100%	86%	100%	29%

Two chloramphenicol resistance genes (Cat and floR) were detected in all (100%) samples from secondary effluents, reservoir, and the outfall, while in samples from tertiary effluents, *cat* gene was highly prevalent (100%) and floR gene was observed in 71%. In samples of the Yarkon stream, the frequencies of both genes displayed the same frequency (86%). *Cat* gene was detected in 29% of samples from the low impacted seawater, whereas, floR gene was not detected in low impact seawater.

The results of our study support the conclusions drawn by Luczkiewicz *et al.*, 2010, who reported that treated wastewater contained up to 90% antibiotic re-sistant *E. coli*. Furthermore, the researchers observed a positive selection of iso-lates with antimicrobial patterns during the wastewater treatment [21]. The re-sults indicate that wastewater treatment plants can be a substantial source for antibiotic resistance bacteria and genes in the receiving aquatic environments. Special concern should be paid to the isolates resistant to 3 or more chemical classes of antibiotics. Urase and Sato (2016) studied the antimicrobial resistance to fluoroquino-lones and third-generation cephalosporins in the Tama river watershed [22]. High occurrence of the multiple resistant bacteria to different classes of newer antimicrobials was reported. The results presented are in agreement with those reported previously, which have shown that the prevalence of ARB in receiving surface water is equal or not lower than their prevalence in treated wastewater effluents [22] [23] [24]. These observations suggest that

wastewater treatment plants are the major source of ARB and ARG, therefore suitable measures should be applied to reduce their discharge to the environment and receiving water bo-dies. This study highlights the importance of wastewater treatment plants in the transmission of ARB and ARG to the environment and especially to receiving streams, rivers and marine waters. The persistence of ARB and ARG in marine waters may enhance the horizontal transmission of ARG to pathogenic bacteria. The results indicate that the Mediterranean coastal waters may be contaminated by ARB and ARG which introduce a serious public health problem for bathersmand seafood harvested from contaminated regions. Improved wastewater treat-ment technologies should be applied to reduce the levels of ARG and ARB re-leased to the environment.

CONCLUSIONS

High occurrence was observed for MDR fecal coliform in wastewater treated effluents. High prevalence of ARG in fecal coliform isolates for *β*-lactam and *tetra-cycline* in treated effluents was recorded. Treatment of effluents by chlorination or UV irradiation may select for specific antibiotic resistance.Wastewater effluents are the source of ARB in surface waters receiving the effluents. Therefore, ARB and ARGs in water bodies can serve as the source of ARGs for pathogenic bacteria.

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