ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Seasonal Variation, Antibiotic Resistance of Some Sewage Bacteria from Hamdan Waste Water Treatment Plant in Basrah City-Iraq

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Abstract

Seasonal variation and antibiotic resistance pattern of some sewage bacteria have been studied during the period of at Hamdan wastewater treatment plant in basrah city-Iraq. The results obtained revealed that there is a seasonal variation in the number of bacteria studied. The bacterial taxa in the present study found to resist several antibiotics. The isolated bacteria are multidrug resistant bacteria. Higher percentage of antibiotic resistance was recorded for bacteria from raw sewage that decreased during the subsequent treatments. Sewage bacteria showed different antibiotic resistance patterns. The effluents to Shatt al-Basrah still contain multidrug resistant bacteria that cause a big health problem through dissemination of multidrug resistant bacteria to the area around.

Introduction

Nowadays, overuse and misuse of antibiotics by human and animals (veterinary medicine) have led to emergence of multidrug resistant bacteria (MDR) which became as an increasing threat to public health. It has been reported that there is a correlation between high concentrations of antibiotics in sewage and elevated levels of resistance to antibiotics by bacteria [1, 2]. Sewage Sewage is an environment ideal for appearing of antibiotic resistant bacteria.

This may be due to the impact of the presence of large amounts of antibiotics that are released to the municipal waste water through human and animal excreta, part of them from hospitals. In addition to that, there are many antibiotic resistant genes have been detected in waste water that may responsible for acquiring sewage bacteria the elevated levels of resistance [3, 4]. In the current work, the seasonal variation, antibiotic resistance profile and patterns of some sewage bacterial taxa at Hamdan waste water treatment plant in Basrah-Iraq have been studied.

Materials and Methods

Sampling Site

Sewage samples were taken monthly during the period of January 2014 to May 2015 from Hamdan waste water treatment plant that receiving sewage from different regions of Basrah through pipelines. Sewage samples were collected from three stations (sites) of the plant: Station 1, the receiving basin (raw sewage), Station 2: sedimentation basin and station 3: the effluents to Shatt Al-Basrah water canal. Sewage samples were collected in disposable plastic containers directly brought to the laboratory for further analysis.

Bacteriological Analysis

Total count of bacteria in sewage: For counting of total bacteria, nutrient agar plates were used. Sewage samples were diluted with physiological sali volume of 0.1ml was spread on nutrient agar plate, incubated in an incubator at 37C for 24 hours.

Isolation and Enumeration of E. coli in Sewage Samples

For the isolation and enumeration of *E. coli*, 0.1 mi of sewage sample was spread on macConkey agar plates, incubated at 37C for 24 hrs. Colonies with morphological characteristics of *E. coli* counted. Colonies were tested by IMVIC test.

Presumptive Isolation of Salmonellae from Sewage Samples

For isolation of salmonellae, xylose lysine desoxycholate medium was used. Colonies with morphological characteristics of salmonellae were counted after 24hrs incubation at 37C.

Isolation of *Staphylococcus aureus* from Sewage Samples

Mannitol salt agar was used. MSA plates were inoculated with 0.1 ml of sewage sample. Plates incubated at 37C for 24 hours. Colonies with golden colour were counted and picked and propagated on nutrient agar plates. A positive reaction with cougulase test indicated *Staphylococcus aureus*.

Antibiotic Susceptibility Testing

Antibiotic susceptibility profiles of bacterial isolates from sewage were determined by standard Kirby-Bauer disk diffusion method [4]. Antibiotics with their respective disk concentrations are as follows:

Amoxacillinclavulanic acid (AMC) (20/10ug), cefazoline (CZ) (30uO) (30ug), cefepime (FEP) 30ug), Cefotaxime (CTX) (30ug) Cifriaxone (CRO) (30ug) cefuroxime (CXM) (30) carrbencillin (PY) (25ug), chloramphenicol (C)(30), ciprofloxacin (CIP) (5ug), norfloxacin (NOR) (10ug), clindamycin (DA) (2ug), kanamycin (K) (30ug), oxacillin (OX) (1ug), rifampin (RA) (5ug) and trimethoprim (TMP,5ug) [5, 6]. The present study reveald that highest bacterial total count for raw sewage samples was recorded during April 2015 ($48x10^3$ cfu/ml).

A decrease in bacterial total count in station 2 (sedimentation basin) and the effluents discharged to Shatt Al-Basrah water canal ($11x10^3$ cfu/ml) for each. Highest total count of Gram-negative fermentative bacteria (E. coli) was $40x10^3$ cfu/ml during April 2015 for raw sewage, lowest $2x10^2$ cfu/ml during January 2014 for the effluents (Fig. 1).

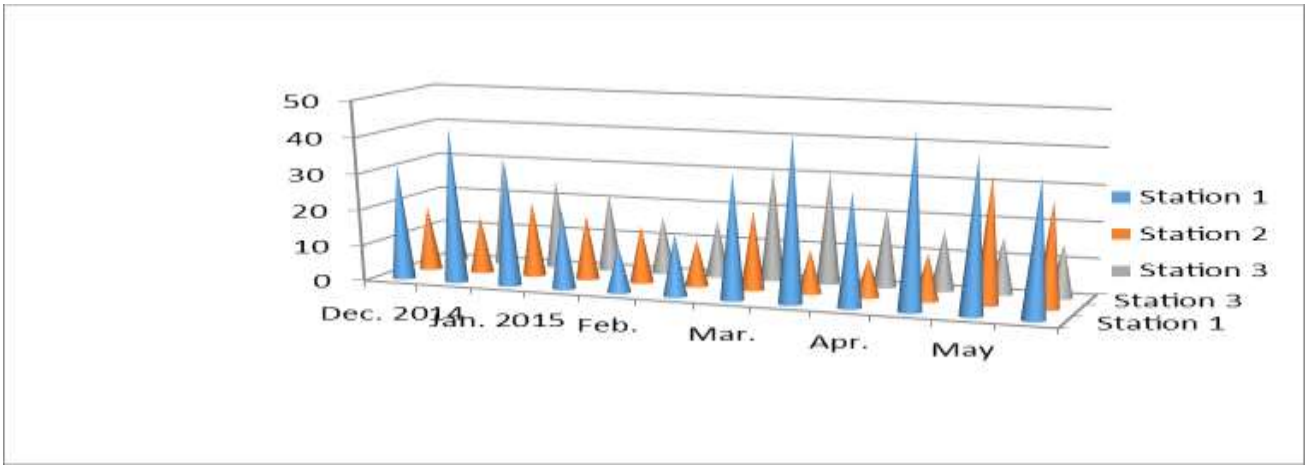

Fig. 1: Total count of sewage bacteria from three stations at Hamdan wastewater treatment plant in Basrah city (1.raw sewage station, 2.Sedimentation basin, 3. The effluents)

Highest total count of Gram-negative fermentative bacteria (E. coli) was $40x10^3$ cfu/ml during April 2015 for raw sewage, lowest $2x10^2$ cfu/ml during January 2014 for the effluents (Fig. 2).

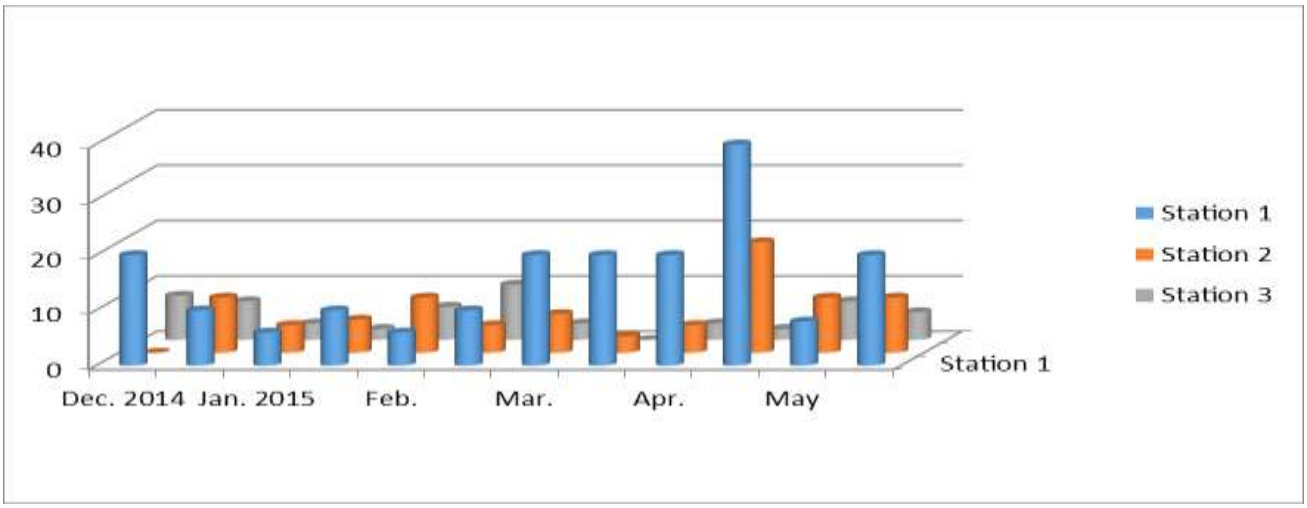

Fig. 2: Count of E. coli from sewage at three stations of Hamdan waste water treatment plant in Basrah city. Station 1: raw sewage, station 2: sedimentation basin, station 3: The effluents

During the period of study, highest count of *Staphylococcus aureus* was recorded at May 5x10^3) for the three stations (Fig. 3).

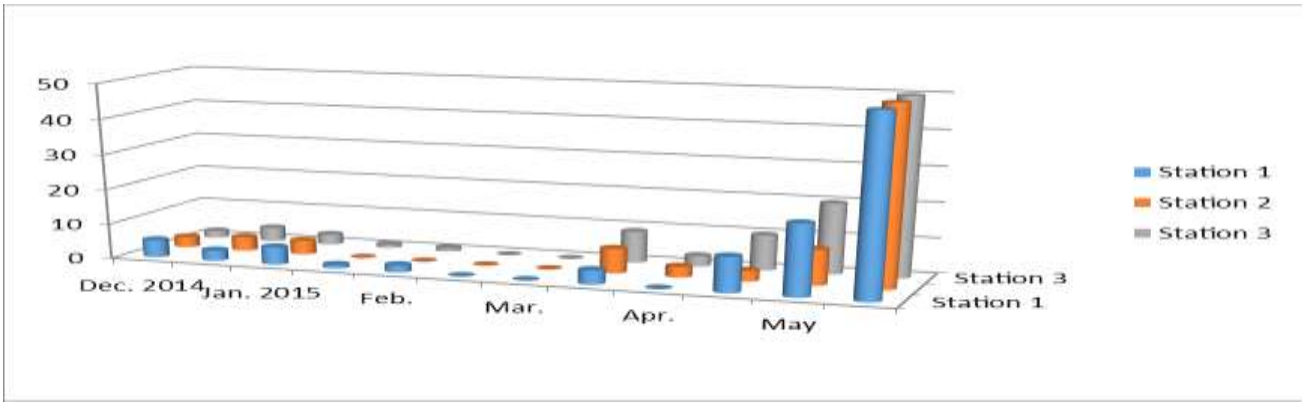

Fig. 3: Count of Staphylococcus aureus from sewage at three stations of Hamdann waste water treatment plant in Basrah city (station 1: raw sewage, station 2: sedimentation basin, station 3: the effluents)

In the present study, for all the stations, antibiotic susceptibility testing of *E. coli* from sewage showed absolute resistance to several antibiotics belonging to different groups. All the isolates resistant (100%) to amoxicillin-clavulanic acid, carbencillin, cefazoline, cefepime, cefotaxime, ceftriaxone, cefuroxime, were sensitive (100%) to chloramphenicol (Table 1). *E. coli* isolates from raw sewage have been shown to be highly resistant to certain antibiotics, i.e., ciprofloxacin and oxacillin. Isolates from sedimentation basin and the effluents showed lesser values of resistance.

Table 1: Antibiotic resistance percentage of *E.coli* isolates from the three stations at Hamdan waste water treatment plant in Basrah city

Antibiotics	Raw sewage %	Sedimentation basin %	The effluents %
Amoxacillin/clavulanic acid	100	100	100
Carbenicillin(Py)	100	100	100
Cefazoline(CZ)	100	100	100
Cefepime(FEP)	100	100	100
Cefotaxime(CTX)	100	100	100
Ceftriaxone(CRO)	100	100	100
Cefuroxime(CXM)	100	100	100
Chloramphenicol(C)	0	0	0
Ciprofloxacin(CIP)	100	70	70
Clindamycin(DA)	100	100	90
Kanamycin (K)	60	60	60
Norfloxacin (NOR)	40	30	0
Oxacillin (OX)	100	30	30
Rifampin (RA)	100	100	100
Trimethoprim (TMP)	80	70	70

In case of *Staphylococcus aureus*, the isolates showed 100% resistance to the following antibiotics for the three stations: amoxicillin-clavulanic acid, cefazoline, cefepime, cefotaxime, cefriaxone, cefuroxime. High percentage of resistance of *S. aureus* from raw sewage (90%, 80%, 70%, 40%) for clindamycin, oxacillin, trimethoprim, kanamycin respectively. A noticed decrease or no resistance towards these antibiotics at sedimentation basin and the effluents (70%, 70%, 70%, 0%) (Table 2).

Table 2: Antibiotic resistance patterns of *Staphylococcus aureus* from three stations at Hamdan waste water treatment plant in Basrah city

Antibiotics	Raw sewage %	Sedimentation basin %	The effluents %
Amoxacillin/clavulanic(AMC)	100	100	100
Carbenicillin(Py)	100	100	100
Cefazoline(CZ)	100	100	100
Cefepime(FEP)	100	100	100
Cefotaxime(CTX)	100	100	100
Ceftriaxone(CRO)	100	100	100
Cefuroxime(CXM)	100	100	100
Chloramphenicol(C)	0	0	0
Ciprofloxacin(CIP)	10	0	0
Clindamycin(DA)	90	70	70
Kanamycin(K)	40	0	0
Norfloxacin(NOR)	10	0	0
Oxacillin(OX)	80	70	70
Rifampin(RA)	100	100	90
Trimethoprim(TMP)	70	70	70

Salmonella isolates from raw sewage showed 100% resistance to the following antibiotics: amoxicillin-clavunalic acid, carbencillin, cefazoline, cefepime, cefatoxime, cefriaxone and cefuroxime. Most of these isolates showed some level of resistance that decreased during subsequent treatments for the other antibiotics studied. Resistance percentage was increased for kanamycin and chloramphenicol while others like ciprofloxacin and norfloxacin, Salmonella isolates became sensitive after waste water treatments (Table3).

Table 3: Antibiotic resistance pattern of *Salmonella* isolates from sewage at Hamdan waste water treatment plant in Basrah city

Antibiotics	Raw sewage %	Sedimentation basin %	The effluents %
Amoxacillin/clavulanic acid (AMC)	100	100	100
Carbenicillin(Py)	100	100	100
Cefazoline(CZ)	100	100	100
Cefepime(FEP)	100	100	100
Cefotaxime(CTX)	100	100	100
Ceftriaxone(CRO)	100	100	100
Cefuroxime(CXM)	100	100	100
Chloramphenicol(C)	60	80	100
Ciprofloxacin(CIP)	10	0	0
Clindamycin(DA)	90	90	80
Kanamycin(K)	80	90	100
Norfloxacin(NOR)	10	0	0
Oxacillin(OX)	90	60	50
Rifampin(RA)	100	100	100
Trimothoprim(TMP)	80	70	70

To evaluate of all the above results, a rate of antibiotic resistance percentage was conducted (Table 4).

Table 4: Rate of antibiotic resistance percentage of the three sewage bacterial taxa in the present study

Station	Rate of antibiotic resistance		
	E. coli	*Staph. Aureus*	*Salmonella* spp.
1	85.33	73.33	81.33
2	77.33	67.33	79.33
3	74.66	66.66	80.0

E. coli showed different patterns of antibiotic resistance. Fourty percentage of the isolates from Hamdan waste water treatment plant showed antibiotic resistance pattern to 13 antibiotics. Some of the isolates resist 14 antibiotics (Table 5).

Table 5: Antibiotic resistance pattern of *E.coli* isolates from sewage at Hamdan waste water treatment plant in Basrah city

Patterns of antibiotic resistance	No. of isolates	No. of antibiotics
DA, CRO, TMP, OX, RA, Py, CXM, CZ, AMC, CTX, FEP	1	11
DA, CRO, TMP, OX, RA, Py, CXM, CZ, AMC, CIP, CTX, FEP	1	12
DA, CRO, TMP, OX, RA, Py, CXM, CZ, AMC, CIP, K, CTX, FEP	4	13
DA, CRO, TMP, OX, RA, Py, CXM, CZ, C, CIP, K, CTX, FEP	1	13
DA, CRO, OX, RA, Py, CXM, CZ, AMC, C, CIP, K, CTX, FEP, NOR	2	14
DA, CRO, TMP, OX, RA, Py, CXM, CZ, AMC, CIP, K, CTX, FEP, NOR	1	14

The dominated pattern that has been shown by 40% of *Staphylococcus aureus* isolates that resist eleven antibiotics is presented in Table 6.

Table 6: Antibiotic resistance pattern of *Staphylococcus aureus* isolates from sewage at Hamdan waste water treatment plant in Basrah city.

Antibiotic resistance patterns	No. of isolates	No. of antibiotics
DA, CRO, RA, Py, CXM, CZ, AMC, CTX, FEP	1	9
DA, CRO, TMP, OX, RA, Py, CXM, CZ, AMC, CTX, FEP	4	11
CRO, RA, Py, CXM, CZ, AMC, C, CIP, CTX, FEP, NOR	1	11
DA, CRO, OX, RA, Py, CXM, CZ, AMC, K, CTX, FEP	1	11
DA, CRO, TMP, OX, RA, Py, CXM, CZ, AMC, K, CTX, FEP	2	12
DA, CRO, TMP, OX, RA, Py, CXM, CZ, AMC, K, CTX, FEP, NOR	1	13

The present study revealed the isolation of one isolate of *Salmonella* resist to fifteen antibiotics. The dominated antibiotic resistant pattern of three isolates of *Salmonella* spp. Was shown in Table 7.

Table 7: Antibiotic resistance patterns of *Salmonella* isolates from sewage at Hamdan waste water treatment plant in Basrah city

Patterns of antibiotic resistance	No. of isolates	No. of antibiotics
DA, CRO, TMP, RA, Py, CXM, CZ, AMC, CTX, FEP	1	10
DA, CRO, OX, RA, Py, CXM, CZ, AMC, K, CTX, FEP	1	11
DA, CRO, TMP, OX, RA, Py, CXM, CZ, AMC, CTX, FEP	1	11
CRO, TMP, OX, RA, Py, CXM, CZ, AMC, C, K, CTX, FEP	1	12
DA, CRO, OX, RA, Py, CXM, CZ, AMC, C, k, CTX, FEP	1	12
DA, CRO, TMP, OX, RA, Py, CXM, CZ, AMC, K, CTX, FEP	1	12
DA, CRO, TMP, OX, RA, Py, CXM, CZ, AMC, C, K, CTX, FEP	3	13
DA, CRO, TMP, OX, RA, Py, CXM, CZ, AMC, C, CIP, K, CTX, FEP, NOR	1	15

Discussion

Hamdan waste water treatment plants in Basrah receive huge body of sewage resulting from the activities of Basrah city (houses and hospitals). Sewage from these sources usually contain intestinal bacteria, a variety of pathogenic bacteria and residues of different antibiotics. During the period of the present study, it can be concluded that there is some observable seasonal variation in the number of sewage bacteria, highest number of bacteria between April and May2015.

It can be attributed to either environmental or certain characteristics of sewage that may related to Biological Oxygen Demand (BOD 5, Total solid particles or perhaps to temperature (unpublished data). Hamdan waste water treatment plants contribute to reduction of the number of bacteria per volume during subsequent treatments. Our finding is consistent with the study of [7] that sewage treatment processs reduce the number of bacteria. The high percentage of antibiotic resistant bacteria present in raw sewage of Hamdan waste water treatment plant contribute to the spread of multidrug resistant bacteria in the around area The present study is conformed with previous results and data that show significantly higher proportion of antibiotic resistant bacteria in raw sewage [8]. *E. coli* in the present study showed high percentage of resistance to B-lactam antibiotics. High frequency of percentage of antibiotic resistance of enterobacteriaceae has been isolated from waste water [9].

In another study on waste water treatment plant revealed a high frequency of resistance of enterobacteriaceae to B-lactam antibiotics through producing class A and class C B-lactamases [10]. It is well known that Sewage is an ideal environment for growth and at the same time may acquist or exchange antibiotic resistant genes and was found to be

favourable for the proliferation of antibiotic resistant bacteria [11]. A previous study mentioned that bacteria in waste water treatment plant harboured various plasmid-borne resistant determinants of all common classes of antibiotics [3]. Sewage usually contains several pathogenic bacteria. Multi-drug resistant pathogenic *E. coli* O157:H7 has been isolated from sewage of Basrah city that harboured 3-4plasmids [12]. In recent study, clinical isolates of *E. coli* from six hospitals in Basrah revealed the presence of bla NDM-1 gene in these isolates with reduced susceptibility to imipenem and meropenem [13]. Genetic study of these isolates showed that 97% of these isolated carried the gene bla NDM-1 gene and 45.9% of -45 carried the gene OXA-48 [14].

The percentage of resistance of sewage bacteria in the current study to certain antibiotics was decreased during subsequent treatments indicate that the treatment process may to somewhat effective in reducing the resistance of bacteria to certain antibiotics but still that waste water treatment plant remain as a source of dissemination of multidrug resistant bacteria to the natural water body of Shatt Al-Basrah river. A decline in resistance levels following sewage treatment was observed by other researchers [15] while others have observed little difference in bacterial resistance levels between raw and treated sewage [7, 16]. Isolation of strains resistant to thirteen to fifteen antibiotics from waste water treatment plant is alarming. The term superbugs can be used to describe these isolates. It is well-known there are five common antibiotic resistant superbugs, carbapenem resistance enterobacteriaceae resistant to several antibiotics in use today and methicillin resistant *Staphylococcus aureus* are examples.

Pattern of antibiotic resistance of sewage bacteria studied at Hamdan waste water plant dominated by isolates resist eleven to fifteen antibiotics. In a comparison study of *E. coli* O157:H7 from river water and sewage, isolates resistant to fifteen antibiotics from sewage water in contrast to 1 isolate resistant to ten antibiotics from river water [12]. It has been found that 40% of *E. coli* strains isolated from sewage were resistant to one or more antibiotics and 9.8% were resistant to more than three antibiotics [17].

References

1. Iwane T, Urase T, Yamamoto K (2001) Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water.

2. Kim S, Aga DS (2007) Potential ecological and human health impacts of antibiotic and antibiotic resistant bacteria from wastewater treatment plants. Journal of Toxicology and Environmental Health. Part B, 10(8):559-573.

3. Szczepanowski R, Linke B, Krahn I, Gartemann K, Gutzkow T, Eichler W, Puhler A, Schluter A (2009) Detection of 140 clinically relevant antibiotic resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. Microbiology, 155: 2306-2319.

4. Xu J, Xu Y, Wang H, Guo C, Qui H, He Y, Zhang Y, Li X, Meng W (2015) Occurrence of antibiotics and antibiotic resistance genes in a sewage treatment plant and its effluent-receiving river. Chemosphere, 119:1379-1385.

5. Bauer AW, Kirby WMM, Sherris JC, Truck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am. J. Cli. Pathol., 43: 493-496.

6. Clinical and Laboratory standards (2012) Performance Standards for antimicrobial susceptibility testing: Twenty-Second Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI Document M 100-S22).

7. Martin da Costa P, Vas-Pires P, Pernardo F (2006) Antimicrobial resistance in *Enterococcus* spp. isolated in inflow, effluent and sludge from municipal sewage water treatment plants. Water Research, 40(8): 1735-1740.

8. Bouki C, Venieri D, Diamadopoulos E (2013) Detection and fate of antibiotic resistant bacteria in wastewater

treatment plants: A review. Ecotoxicology and environmental safety.

9. Reinthaler FF, Posch J, Feierl G, Wust G, Haas D, Ruckenbauer G, Mascher F, Marth E (2003) Antibiotic resistance of *E. coli* in sewage and sludge. Water Research, 37: 1685-1690.

10. Amador PP, Fernandes RM, Prudencio Mc, Barreto MP, Durate IM (2015) Antibiotic resistance in waste water: Occurrence and fate of Enterobacteriaceae producers of class A and class C B-lactamases. J. Environ. Sci. health Subst. Environ. Eng., 50(1):26-39.

11. Jury KL, Vancov T, Stuetz RM, Khan SJ (2010) Antibiotic resistance dissemination and sewage treatment plants. Current Research, Technology and Education Topics in Applied Microbiology and Microbiol technology. A Mendez-Vilas (ed.): 509-519.

12. Al-Hamdani M, Shaban AD (2015) Isolation, Antibiotic resistance patterns and plasmid profile of *E. coli* O157:H7 from environmental samples in Basrah Governorate-Iraq. International Journal of Pharmaceutical and Medical Research, 3(2): 61-64.

13. Abas IJ, Al-Hamdani MA (2017a) New Delhi Metallo-B-Lactamase 1 (NDM-1) producing E. coli in Basrah hospitals-IRAQ. European J. Biomedical and Pharmaceutical Sciences, 4(2): 102-107.

14. Abas IJ, Al-Hamdani MA (2017) Barbapenem resistance-Determining genes among multi-drug resistant bacteria isolates from clinical samples in Basrah Governorate. European J. Biomedical and Pharmaceutical Sciences 4(6): 60-72.

15. Garcia S, Wade B, Bauer C, Craig C, Nakaoka K, Lorowitz W (2007) The effect of wastewater treatment on antibiotic resistance in *Escherichia coli* and *Enterococcus* sp. Water Environment Research, 79(12):2387-2395.

16. Espigares E, Bueno A, Espigares M, Galvez R (2006) Isolation of *Salmonella* serotypes in wastewater and effluent: Effect of treatment and potential risk. International Journal of Hygiene and Environmental Health, 209(1):103-107.

17. Reinthaler FF, Posch J, Feierl G, Wust G, Haas D, Ruckenbauer G, Mascher F, Marth E (2003) Antibiotic resistance of *E. coli* in sewage and sludge. Water Research, 37(8):1685-1690.

