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Isolation and identification of Multidrug-Resistant (MDR) bacteria from wastewater in some hospitals in Basrah

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Abstract

Wastewater samples were collected for some hospitals and nearby houses in Basra Governorate. Fifteen samples of wastewater were collected from some hospitals in Basra Governorate. Two samples were taken from each hospital, including wastewater, before and after treatment. An effluent water sample was taken from the homes near these Hospitals ,and then 47 bacterial isolates were isolated from these samples and, The results showed that the number of bacterial isolates before sewage water treatment at a percentage of (44.68%) , the number of bacterial isolates after sewage water treatment at a percentage of (34.04%) nd the number of bacterial isolates samples from the effluent water from a domestic house near a hospital house at a percentage of(21.27%). growth-negative isolates at a percentage 93.61 % and growth-positive samples isolates at a percentage (6.38%). 16 samples were diagnosed with multidrug resistance (MDR) at a percentage (34.04%) of the total isolates were positive and negative in growth bacterial sample , represented by Burkholderia Ciencia, (2.12%), Pseudomonas aeruginosa. (10.63%), Klebsella Pneumoniumea,(8.51%), Staphylococcus aureus, (4.25%), Enterobacter aerogenes and Staphylococcus Pseudintermedius by (2.12%), Escherichia coli (4.25%). The results of the study showed that all isolates Gram-positive of were characterized by their resistance to multiple antibiotics such as Trimethoprim, Benzylpenicillin, and Oxacillin; the current study also showed the resistance of the Gram-negative bacterial isolates to Tetracycline, Trimethoprim/ Sulfamethoxazole, Gentamicin, Aztreonam, Oxacillin Amoxicillin , Doxycycline. The results also showed the sensitivity of all Gram- positive isolates to the Ciprofloxacin, Gentamicin, Vancomycin antibiotics. At the same time, the results also indicated that some Gram-negative bacterial isolates are sensitive to Piperacillin, Meropenem, Ciprofloxacin and Gentamic antibiotics.

Keywords: Multidrug-Resistant, MDR, wastewater

1. Introduction

The resistance of bacteria to antibiotics is considered one of the most important problems that the world faces in terms of health and economy. Many bacterial strains have acquired antibiotic-resistant properties, as a result of the excessive and widespread use of antibiotics in treatment. Antibiotic treatment is the most important means of controlling some cases of bacterial infection, and that bacteria are resistant to antibiotics Vitality is responsible for thousands of deaths each year (WOH.,2022).

The hospital environment is one of the suitable environments for the growth and living of such multi-antibiotic-resistant bacteria, which reduces the treatment options available to them and becomes more expensive. The access of these multiresistant bacteria to hospital sewage is another serious problem (Murray et *al*., 2022).

Wastewater in hospitals is a major source of environmental pollution, especially in developing countries in the world, where there are no treatment plants in all hospitals, and even if they exist, they are not equipped to treat it effectively and may be out of work, and wastewater containing many neighborhoods is produced. Microscopic medical and non-medical human activities, in addition to medical waste and waste in hospitals. And that this waste in the hospital is a threat to public health and environmental balance if left untreated. This infectious and pathogenic waste leads to the spread and transmission of infectious diseases. Polluted wastewater that is discharged from Hospitals are in many environmental hazards. (Zhang et *al*., 2020).

Hospital wastewater may contain many potentially hazardous substances including microbiological pathogens, radioisotopes, disinfectants, drugs, chemical compounds, and pharmaceuticals. Hospitals excrete many unwanted potential pathogens such as antibiotic-resistant bacteria and viruses. These problems vary in terms of the activity and nature of hospitals and also the chemicals used in hospitals are potential sources of water pollution, especially through the sewage system (Pratibha et *al*., 2014)

Hospitals are an indisputable discharge source for many chemical compounds into the aquatic environment due to laboratory efficacy or drug excretion into wastewater or into the environment that can cause environmental water pollution and human health problems. (Addae-Nuku.,2022)

2. Materials and Methods

2-1 **Sample Collection**: Fifteen samples of 47 isolates were collected from the sewage water of some hospitals and nearby houses in Basra Governorate, then transferred to the laboratory and placed directly on the culturing media to be incubated for 24 hours at 37° C.

2-2 **Phenotypic Diagnosis**: The phenotypic characteristics of the isolated colonies were studied after culturing the bacterial isolates and purifying them on culture media and after cultivating them on MacConkey agar medium to distinguish between Gram-negative and Gram-positive bacteria and those that

are lactose fermenting and non-lactose fermenting bacteria. Then, they were examined under an optical microscope to investigate the shape, arrangement and colours of the cells according to their interaction with Gram stain

2-3 **Biochemical Tests**: Some tests were conducted on Gram-positive and Gramnegative bacteria, such as the catalase test, the oxidase test, the motility test by hanging drop method, and the medium mannitol test.

2-4 **Drug Sensitivity Test**: The sensitivity of bacterial isolates to various antibiotics was tested based on CLSI (2020) according to the standard Kirby- Bauer disk diffusion method.

3. Results and Discussion:

The current study collected sewage water samples from some hospitals and nearby houses in Basra Governorate. Fifteen samples of wastewater were collected from some hospitals in Basra Governorate. The close circles were grown on Nutrient Agar medium, and then 47 bacterial isolates were isolated from these samples and planted on Maconkey Agar and Blood agar base medium. The results showed that the number of isolates of negative growth samples is (44) ,93.61% and positive growth samples are (3) , 6.38 % , as in Table (1-1)The results showed in Table (1-2) that the percentage of the presence of bacteria in the wastewater before treatment is (44.68%) , number of bacterial isolates in the l wastewater after treatment (34.04%), and the percentage of the number of bacterial isolates in the water In the homes near hospitals, which was at a rate of (21.27%).The results showed that these percentages are commensurate with the nature of the environment in hospitals, the number of bacterial isolates in the wastewater before treatment was greater than the number of bacterial isolates in the wastewater after treatment due to the arrival of a lot of medical waste and waste contaminated with chemicals to the wastewater , and then when subjected to treatment, the number of bacterial isolates decreased. Wastewater is often discharged from hospital facilities and is the source of many sensitive or antibioticresistant bacteria in the sewage system without prior treatment, which contributes to an increase in the concentration of drugs as well as the number of bacteria Antibiotic resistance and antibiotic resistance genes in municipal wastewater ,and then their access to domestic effluent water (Al-Enazi.,2016).

Table (1-1) shows the number of antibiotic-resistant study samples

Total samples	Positive Growth samples	Negative Growth samples	Multidrug resistant sample
47	3	44	16
%100	%6.38	% 93.61	)%33.43(

Table (1-2) shows the number of bacterial isolates in wastewater before and after

N	The sampling site	Number of bacterial isolates from pre-treatment wastewater	Number of bacterial isolates from after treatment wastewater	The Number of isolatesfrom houses near to hospitals
1	Al-Sadr AL talemi Hospital	5 (10.63%)	3)%1..8 (	2 (4.25%)
2	Al-Shifa Hospital	4 (8.51%)	3)%1..8 (	2 (4.25%)
3	Qurna l Hospital	4 (8.51%)	3)%1..8 (	2 (4.25%)
4	Al-ZubairHospital	4 (8.51%)	.)%3...(	2 (4.25%)
5	Al-FawHospital	4 (8.51%)	.)%3...(	2 (4.25%)
	total	21(44.68%)	16(34.04%)	10(21.27%)

The results showed in Table (1-3) that the number of multidrug-resistant isolates in the study samples of wastewater before and after treatment and effluent water was 16 isolates at a percentage 34.04% of the total number of study samples, as they were resistant to at least one antibiotic in three different categories of antibiotics. The number of Burkholderia Cepcia bacteria 1, 2.12%, 5isolates from Pseudomonas aeruginosa 10.63%, 4 isolates from Klebsella pneumoniumea 8.51%, 2 isolates from Staphylococcus aureus, 4.25%, and one isolate each from Enterobacter aerogenes and Staphylococcus pseudintermedius a percentage. 2.12% and two isolates of Escherichia coli. 4.25%, antibiotic sensitivity was tested using the vitk2 device.

Table (1-3) shows the number of antibiotic-resistant bacterial species

N	Type	number of isolates	percentage
1	Burkholderia Cepcia	1	2.12%
2	Pseudomonas aeruginosa	5	10.63%
3	Klebsilla Pneumoniae	4	8.51%
4	Staphylococcus aureus	2	4.25%
5	Enterobacter aerogenes	1	2.12%
6	Staphylococcus pseudintermedius	1	2.12%
7	Escherichia coli	2	4.25%
	Total	16	(34.04%)

Table (1-4): Phenotypic diagnosis and biochemical tests of multi-antibiotic resistant bacteria

N	Diagnosis	Cell shape	Macon key	Lactose fermentation	Gram stan		
1	Burkholderia Cepia	bacilli	+	+	_	_	+
2	Psedomnas aerginosa	bacilli	+	-	-	+	+
3	Escherichia coli	Rod	+	+	_	_	+
4	Klebsiella Pneumoniae	bacilli	+	+	_	_	
5	Psedomonas Putida	bacilli	+	_	_	+	+
6	Enterobacter aeroges	bacilli	+	+	_	_	
7	Aeromonas sboria	bacilli	+	+	_	_	
8	*ylococcus aureus*	Cocci	_	_	+		+
9	*Staphylococcus pseudintermedius*	Cocci	_	_	+		+
01	*Aeromonas hydrophila*	bacilli	+	+	_	_	

4. **Antibiotics Sensitivity Test Results for Isolated Bacteria**

The results of the study showed that all isolates Gram-positive of were characterized by their resistance to multiple antibiotics such as Trimethoprim, Benzylpenicillin, Oxacillin ,Trimethoprim/ Sulfamethoxazole, which is consistent with (AL Mozfer .,2016) The cause of resistance may be attributed to the presence of efflux pumps that help them flush antibiotics out of the bacterial cell (Papkou et al., 2020), and the bacteria's production of biofilms is an essential factor in increasing their multidrug resistance (Senobar Tahaei et al., 2021).

The current study also showed the resistance of the Gram-negative bacterial isolates to Tetracycline, Trimethoprim/ Sulfamethoxazole, Gentamicin, Aztreonam, Oxacillin Amoxicillin , Doxycycline , antibiotics in varying strengths. The resistance may be due to several reasons, including the presence of efflux pumps in bacteria, as well as their production of β-lactamases enzymes, which dismantle the β-lactams ring and inhibit the action of antibiotics belonging to the penicillins and cephalosporins (Paltansing, 2015).

The results also showed the sensitivity of all Gram-positive isolates to the Ciprofloxacin, Gentamicin, Vancomycin antibiotics, while the results also indicated that some Gram-negative bacterial isolates are sensitive to the Piperacillin, Meropenem, Ciprofloxacin and Gentamic antibiotics (Abd Al Wahid and Abd AlAbbas, 2019) and (AL Mozfer .,2016)The reason for this difference in the sensitivity or resistance of bacterial isolates can be explained by the extent to which these isolates are exposed to factors that lead to the emergence of resistance, including the indiscriminate use of antibiotics for long periods without following medical instructions (Al-Tamimi et al., 2022).

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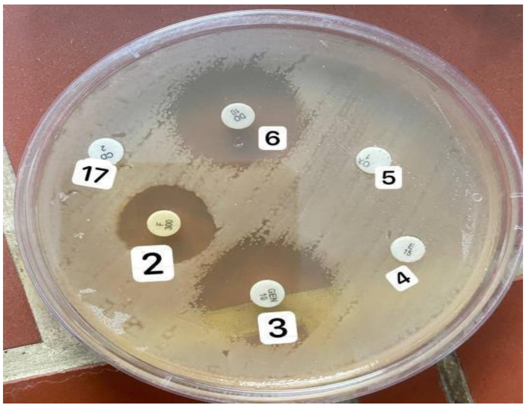
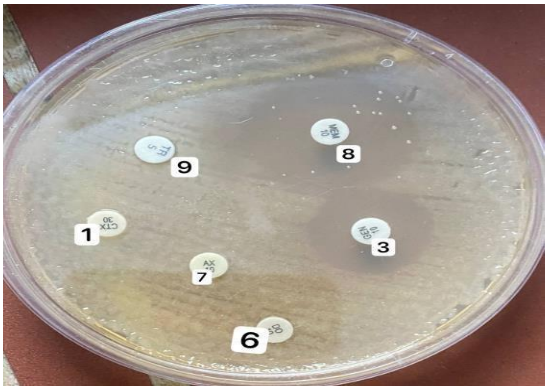
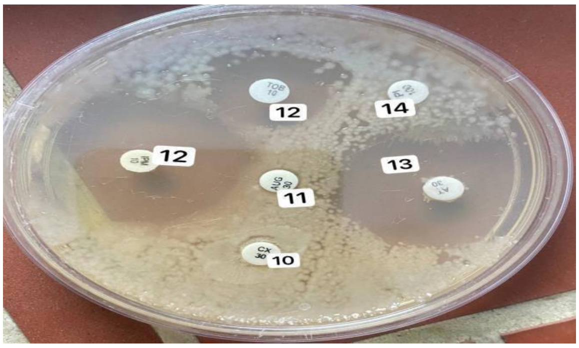


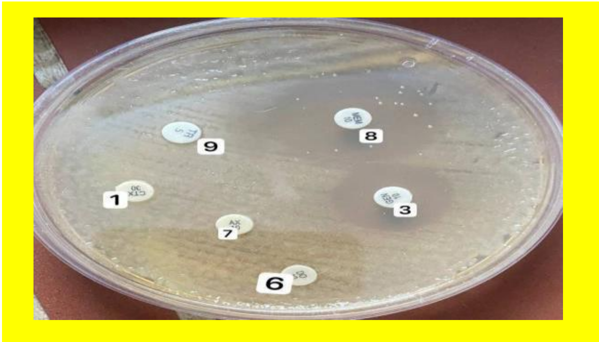
Staphylococcus aureus



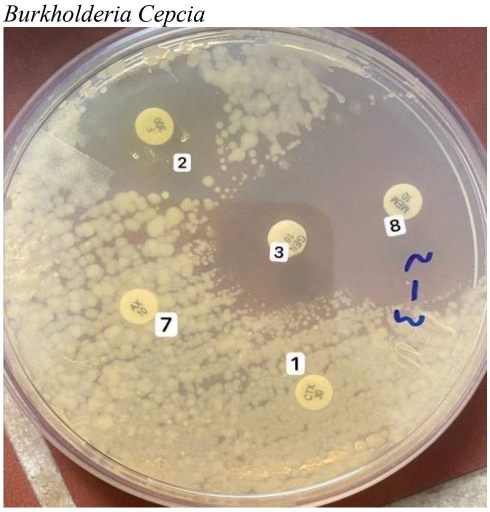
Enterobacter aeroge



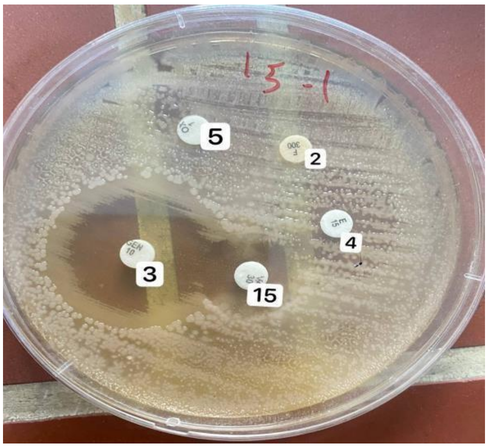
Burkholderia Cepcia



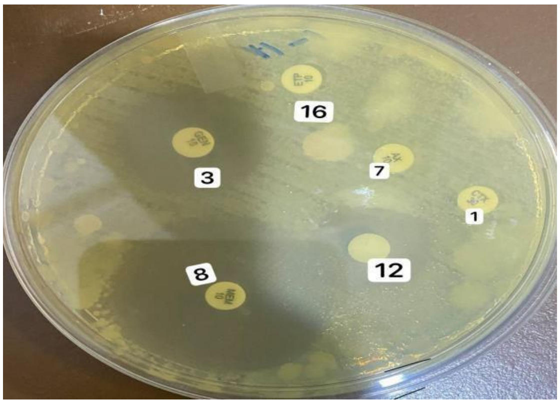
Klebsiella Pneumonume



Escherichia coli



Staphylococcus pseudintermedius



Psedomnas aerginosa

Figure 1 shows drug sensitivity testing on Mueller-Hinton Agar plates cefotaaxime=1, Nitrofurantoin=2, Gentamicin=3, Erythromycin=4, Oxacillin=5, Doxycycline=6, Amoxicillin =7, Meropenem=8, Trimethoprim=9, Cefoxitin=10, Amoxicillin Clavulanic acid=11, Imipenem=12, Aztreonam 13,Piperacillin=14, Vancomycin=15, Ertapenem=16,clindamycin=17, Table (1-5) Drug sensitivity test for multidrug resistant isolates

NO /AN	MOX	LEV	BZ	MIN	NOR	cfp	Cef	AT	TI	R	FA	PRI	AX	OX	CTX	DO	ERM	MEM	DC	E	GEN	F	TR	TOB	CXT	IMP	AUG	AV	ETP
1	R		R	I		R	R	R	R	R		R	R					I			I			S		S	R		R
2	R	R	R											R		S			R	R	S	S		S				S	R
3		R	R											R		S			R	R	S	S		S				S	R
4						R	R						R	R				S			S		R			S			
5						R	R						R	R				S			S		R			S			
6						R	R						R	R				S			S		R			S			
7						R	R						R	R				S			S		R			S			
8						R	R						R	R				S			S					S			
9					R	S	S					S	R					S			S	I	S		S				I
10					R	S	S					S	R					S			S	I	S		S				I
11					R							R	R					R			S	R			R	I			
12					R							R	R					R			S	R			R	I			
13					R							R	R					R			S	R			R	I			
14					R							R	R					R			S	R			R	I			
15		S		R	R	R	R	R	R			R	R			R	S	S			S	S	R	S	R	I			S
16	S	S	R								R			R					R	R	S	R		S				S	R

R= Resistant, I=Intermediate, S= Sensitive

References:

Abd Al Wahid, Z. and Abd Al-Abbas, M. J. (2019). Detection of E. Coli Strains Isolated from Water Sources and Diarrhea Cases by Random Amplified Polymorphic DNA in Basrah Governorate. J International Journal of Sciences, 8, 68-83.

Addae-Nuku, D.S., Kotey, F.C., Dayie, N.T., Osei, M.M., Tette, E.M., Debrah, P. and Donkor, E.S. (2022). Multidrug-Resistant Bacteria in Hospital Wastewater of the Korle Bu Teaching Hospital in Accra, Ghana. Environ Health Insights. Oct 22;16:11786302221130613. doi: 10.1177/11786302221130613. PMID: 36311334; PMCID: PMC9597020.

Al-Enazi M.S. Evaluation of wastewater discharge from Al-Sadr teaching hospital and its impact on the Al-Khorah channel and Shatt Al-Arab River in Basra City-Iraq. Evaluation. 2016;6(12)

Al-Muzaffar, Suha Muhammad Ali Muhammad Saeed. (2016). Estimation of some environmental and bacteriological factors of wastewater in Basra Governorate/Iraq. Master Thesis, Department of Life Sciences, University of Basra.

Al-Tamimi, M.; Albalawi, H.; Shalabi, M.; Abu-Raideh, J.; Khasawneh, A. I. and Alhaj,
F. (2022). Cefixime and cefixime-clavulanate for screening and confirmation of extended-spectrum beta- lactamases in Escherichia coli. Annals of Clinical Microbiology and Antimicrobials, 21(1), 1–7.

Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A.,
Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran,
E. A. P., McManigal, B., ... Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. In The Lancet (Vol. 399, Issue 10325, pp. 629–655). https://doi.org/10.1016/S0140-6736(21)02724-0

Papkou, A.; Hedge, J., Kapel, N.; Young, B. and MacLean, R. C. (2020). Efflux pump activity potentiates the evolution of antibiotic resistance across S. aureus isolates. Nature Communications, 11(1), 3970.

Paltansing, S. (2015).Antimicrobial resistance in Enterobacteriaceae: characterization and detection (Doctoral dissertation, Leiden University). (pp 155).

Senobar Tahaei, S. A.; Stájer, A.; Barrak, I.; Ostorházi, E.; Szabó, D. and Gajdács, M. (2021). Correlation between biofilm-formation and the antibiotic resistant

WHO, World Health Organization (2022). Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda. Guideline, 583pp.

Zhang, L., Ma, X., Luo, L., Hu, N., Duan, J., & Tang, Z. (2020). The Prevalence and Characterization of Extended-Spectrum β -Lactamase- and CarbapenemaseProducing Bacteria from Hospital Sewage , Treated E ffl uents and Receiving Rivers.