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Agricultural Quality Evaluation of Wastewater, Used in Yemen Vegetables Production

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Abstract: The aim of this study is to evaluate the quality and suitability of canal wastewater, Shallow wells and Ponds, in Bani Al Harth area of Sana'a Yemen, when used to irrigate vegetables production. This assessment was done by the Physicochemical and Microbiological characterizations and ranking the isolated Enterobacteriaceae. The physicochemical parameters of both water tested (pH, EC, TDS, NO_3 and SO_4) showed results in agreement with the standards of FAO and WHO with, respectively, averages of 6.93, 2047 µS/cm, 1305, 10.9 mg/L and 57.3 mg/L, but COD mg/l, BOD5 mg/l and turbidity value exceeded even the maximum value for the allowable in wastewater irrigation use with the main of 362, 93.2 and 231 NTU. Microbiological analyses showed higher counts in all tested samples, with an average $1.3x10^7$ CFU/ml, $8.1x10^6$CFU/ml, $1.3x10^7$ CFU/100ml, $5.9x10^{5}$' CFU/100ml, $5.4x10^2$CFU/100ml, $1.1x10^4$ CFU/100ml, $4.2x10^3$CFU/ml and $6.7x10^2$CFU/ml for, respectively, Heterotrophic Plate Count at 22 °C & 37 °C, total coliforms, fecal coliforms, *Staph.* spp, *Vibiro* spp, yeasts and moulds. Also *Salmonella* spp was detected in all tested samples. Enterobacteriaceae identification results showed the presence of *Escherichia coli* (25%) then *Enterobacter* genus (21.4%) with three species *E. aerogenes* (50%), *E. Amnigenus* (33%) and *E. intenrmedius* (17%). Other genus *Klebsiella* (18%), *Citrobacter* (14.3%), *Serratia* (10.7%) and *Proteus* genes (10.7%); were also observed. On the other hand, when the microorganisms isolates were tested versus antibiotic, they showed be highly susceptible to Gentamicin and Cefotaxime; while, three isolates were observed resistant to 25µg of Amoxicillin+Ac clavulanic. And, one isolate was Cefalotin resistant. We, therefore, conclude that both waters samples examined did not meet bacteriological quality standards. Thus Sana'a wastewater effluent and its agricultural reuse under these conditions can be considered illegal.

Key words: Wastewater · Suitability · *Enterobacteriaceae* · Antibiotic Susceptibility

INTRODUCTION

Wastewater reuse can constitute one way to serve agriculture in arid and semi-arid area which suffers from a lack of food production. It's usually used in developing countries, but in the same time it represents one from healthy concern main problems because microorganism's content of the water could contaminate fruits and /or vegetables. This can constitute a potential public health risks associated with wastewater use [1-3]. The health risk associated with wastewater has been major deterrent when it's applied in irrigation. The municipal wastewater used in agriculture requires a careful monitoring of a range of hygiene Parameters [4]. Many works have well documented the potential health risks and impacts on environmental resulting from wastewater use for irrigation [5, 6]. Various types of wastewater as urban, industrial, agricultural and washout from the hospitals were received to wastewater treatment plant as such, wastewater is rich in organic matter which is an important source of organic matter therefore, wastewater is habitat for many groups of microorganisms especially bacteria and fungi [7, 8].

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Studies carried out in Morocco [9], in Italy [10] and in many other countries have reported that microbial pollution must attach with wastewater. As example, water shortages are affecting the Yemen country. Because the capital Sana'a city of the Yemen Republic, has year drought since 1980. So, In the Bani Al harth area, with a total surface of 269 km^2 and, situated near Sana'a city, it's considered an important area to vegetables production. Scarcity and high cost of fresh water in that region caused reused effluent from treatment plants for agriculture. Although, wastewater use in the world—there is poor Wastewater treatment in Sana'a. The treatment system is operating overloaded because the applied design parameters have changed due to the drought period in Yemen which led to low water consumption causing higher concentrations of pollutants in the incoming wastewater. So, the existing BOD_5 observed is higher than 1000mg/L which is two times superior *versus* the design value of 500mg/L. This resulted in an overloading by 200% higher than the design loading [11, 12]. Many health and agronomic risks were appeared. The studied area depends upon the sewage water in about 95%, 5% from ground water only to irrigate the cultivated areas [13]. Among reported cases, most animals in Bani Al Hareth in Sana'a suffering from intestinal diseases like: swelling of stomach, liver calcification, changes the taste of milk and intestinal worm's diarrhea and mouth blister. Also the farmers of these areas suffering from derma logical diseases and mouth blister because they didn't wear gloves when monitored wastewater irrigation [14] and our farmer Questionnaire during the period of analysis showed same symptoms. Diarrhea in new hosts has been caused by enteric pathogens, including *Salmonella spp*, *Shigella spp*, *Vibrio cholerae* [15].

In all, it is noticed that, microbiological aspects of wastewater are not investigated in Yemen and knowledge on the distribution of pathogenic bacteria and fungi is also absent relatively the wastewater reuse. Several countries have existed at present legislation or guidelines for regulation the range of microbial treated final effluent the basis of risk to the public health and the environment, however, in Yemen; such guidelines are not existed up to date.

MATERIELS AND METHODS

Sampling: Between June 2012 and November 2012 one hundred and sixty five water samples were collected from three water sources used in Bani Al Hareth area at different times. The study included wastewater canal, shallow wells and ponds samples along the great wastewater canal of Sana'a Wastewater Treatment Plant effluent (SWTP). Five sampling points were chosen for the samples collection from wastewater canal. Then, the samples were selected from the point that serving "is usually the point where most farmers use" to irrigate vegetables which eaten uncooked in different distance from (SWTP). And, samples were divided into five groups (G1, G2, G3, G4 and G5) in order to perform the effect of distance from the treatment plant between the groups; on the chemical and biological characteristics of treated wastewater used in irrigation. So, G1 is the samples average at outlet of treatment plant and G2 at about 200m of the outlet of the treatment plant, G3 represented branch canal which support by Pumping machine from the main canal in Bait Al-Hillalli about 1200m away from the plant, G4 is average of samples at Bait Al-khawi situated at about 3500m away the plant and G6 the average of samples at Bait Haron about 5000 -5600km from the plant. Six shallow well sampling locations were selected in this study and concern the region around the wastewater canal. The sampling points were distributed all over the study area to ensure appropriate spatial coverage of the surface water. All the sampling points were pumped wells. The average depth of the sampled wells is 30m. The W1, W2, W3, W4and W5, are situated far from the plant about, respectively, 300m, 200m, 500m, 3500 and 1500m from canal. The representing by W6 is situated about 4500 from plant 350m from the main wastewater canal and all the wells sampled within one km to 4km far of a wastewater plant drain in Bani A-Harth area.

Concerning ponds water, samples were collected from three various places representing the study area; from what often freshly harvested vegetables are washed to remove soil before their transfer to markets. The P1 considered the first pond in the farm using contaminated shallow well and P2 the Second pond in farm which uses wastewater for vegetables irrigation in Bait Al-Hillalli. And the third pond P3 in the farm uses shallow well to irrigate in Bit Al-Khawi. For the quality analysis, the samples collection was immediately transported to the microbiology Laboratory in Sana'a university and lab of the local company for water and sanitation (SWSLC) for further analyses. All samples were stored at less than 4°C until analysis.

Hygienic Quality Characterization: Physicochemical characterization based on field measurements and laboratory, including temperature, pH, electrical conductivity, total dissolve sold TDS, Turbidity, COD, BOD5, NO_3 and SO_4; were carried out. Physicochemical were evaluated in according to Standard Methods for the Examination of Water and Wastewater [16]. Also Microbiological characterization including Heterotrophic Plate Count (H.P.C at 22°, 37°C), total and fecal coliforms., *Salmonela spp*., yeasts and molds were monitored as per [16]. Whilst, *Vebiro spp*. were determined as per [16, 17] and Staphylococci were isolated on Mannitol Salt Agar at 30°C, from yellow or red colonies (typical staphylococci colonies as described by Difco Manual [17] and Faria *et al*. [18].

Isolation and Identification of Enterobacteriaceae: After an overnight cultivation, bacteria colonies were transferred from several selective media. Sub-cultures were made, to get single colonies many times until purified. All the isolates bacteria were subjected to Gram stain and oxidase reaction. Bacteria isolates identification was conducted by determination of the biochemical reaction profile using commercial API 20E kit (Biomerieux, Marcy l'Etoile, France).

Bacteria Antibiotic Sensitivity: Some bacteria isolates were tested for their sensitivity to certain known antibiotics. And the antimicrobial sensitivity was tested to the *E.coli, Enterobacter* and *Klebsiella* isolates and was performed using agar disk diffusion method according to CLSI [19]. Then, the following antibiotic disks (Oxoid Ltd., England): AMP: Ampicillin 10µg, AMX: Amoxicillin 10µg, AMC: Amoxicillin+Ac clavulanic 20µg, CTX: Cefotaxim 30µg, CF: Cefalotin 30µg and GM: Gentamicin 10µg were used to monitor and evaluate these bacteria's sensitivity.

RESULTS AND DISCUSSION

Physicochemical Characterization: Results of physicochemical analysis of water samples from different sources are presented in Table 1. Mean values relatively to the temperature were ranged between 20 to 22°C, while pH was observed in agreement with FAO recommendations with values ranged between 6.54 to 7.6. Similar results were reported by Mutengu *et al*. [1] who found wastewater pH value between 8.1 and 7.3; also have explained that irrigation water with a pH outside the normal range causes a nutritional imbalance thereby affecting plant growth.

Electrical Conductivity (EC): values obtain in wastewater, Pond's water and wells water varied from 2267 to 2150, 2270 to 2203 and 1920 to 1423 µS/cm, respectively with an average 2047 µS/cm, the highest value was obtained in P2, however the lowest values was showed in W2. All wastewater group were with line value of FAO [20] recommend. According of this limited, the effluent could be caused moderate problems.

Total Dissolved Solids (TDS): TDS determined in samples from canal wastewater, ponds water and wells values were observed varied, respectively, from 1467 to 1397 mg/l, 1449 to 1339 mg/l and 1223 to 910 mg/L. with 1305 mg/L as average. The highest value was obtained with G3 sample. However, the lowest value was showed in W2. This value was in line with the slight to moderate of FAO [21] recommendations of water quality designated for irrigation which is 450-2000 mg/l, but exceed the result obtain by AL-Jasser [21] which is from 660 to 1220mg/l.

Table 1: Physicochemical characterization of the water samples

Sits	Tem °C	pH	EC µS/cm	TDS mg/L	BOD_5 mg/L	COD mg/L	Turbidity NTU	NO_3 mg/L	SO_4 mg/L
G1	22.1	7.03	2267	1440	128	438	430	8.03	66.0
G 2	22.2	7.63	2217	1423	116	414	415	9.62	85.3
G 3	22	6.60	2255	1467	140	546	440	13.3	65.3
G4	22.2	6.90	2150	1397	96	408	431	13.5	94.6
G 5	22.2	7.00	2216	1402	113	396	390	15.9	102
P1	21.5	6.54	2203	1389	37	127	380	8.2	140
P2	22.3	6.91	2270	1449	78	491	393	9.6	96
P3	22.7	6.74	2257	1422	36	79	350	8.3	145
W1	21.2	6.88	1899	1211	-	-	0.44	12.4	1.4
W2	22	7	1423	910	-	-	0.90	8.4	1.5
W3	20	6.74	1884	1205	-	-	0.95	15.9	1.5
W4	22.1	7.12	1850	1157	-	-	0.91	13.8	0.4
W5	22	6.80	1920	1223	-	-	0.91	7.3	1.5
W6	21.9	7.09	1844	1172	-	-	0.90	8.4	0.9
Average	22.0	6.93	2047	1305	93.2	362	231	10.9	57.3

G1-G5 point of wastewater canal, P1-P3 ponds, W1-W6 shallow well

Table 2: Estimated counts of pathogens contamination of canal wastewater, shallow wells and ponds samples

	Bacteria								
	HPC UFC/ml								
Sits	22°	37°	TC UFC/100ml	FC UFC/100ml	*Staph. spp* UFC/100ml	*Vibiro spp* UFC/100ml	*Salmonella spp*	Yeasts UFC/ml	Molds UFC/ml
G1	$3.1x10^{7}$	$1.6x10^{7}$	$3.3x10^{7}$	$3.2x10^{5}$	$1.7x10^{3}$	$2.1x10^{4}$	+	$4.6x10^{4}$	$2.4x10^{2}$
G2	$3.0x10^{7}$	$2.0x10^{7}$	$8.9x10^{6}$	$2.4x10^{5}$	$1.4x10^{3}$	$1.8x10^{4}$	+	$2.8x10^{3}$	$1.1x10^{2}$
G3	$2.8x10^{7}$	$1.4x10^{7}$	$3.7x10^{7}$	$1.3x10^{6}$	$1.3x10^{3}$	$1.3x10^{4}$	+	$1.2x10^{3}$	$1.6x10^{3}$
G4	$4.2x10^{7}$	$3.5x10^{7}$	$4.3x10^{7}$	$1.4x10^{6}$	$6.0x10^{2}$	$4.7x10^{4}$	+	$2.3x10^{3}$	$6.3x10^{2}$
G5	$3.0x10^{7}$	$1.2x10^{7}$	$3.0x10^{7}$	$5.2x10^{5}$	$1.1x10^{3}$	$2.1x10^{4}$	+	$8.9x10^{2}$	$8.5x10^{2}$
P1	$7.1x10^{6}$	$1.7x10^{6}$	$4.8x10^{6}$	$1.1x10^{6}$	$3.3x10^{2}$	$3.0x10^{2}$	-	abs	$3.0x10^{2}$
P2	$1.2x10^{7}$	$1.5x10^{7}$	$1.8x10^{7}$	$2.7x10^{6}$	$6.7x10^{2}$	$2.0x10^{4}$	-	$2.3x10^{2}$	$5.3x10^{3}$
P3	$2.3x10^{5}$	$3.2x10^{5}$	$1.3x10^{7}$	$7.4x10^{5}$	$3.3x10^{2}$	$1.7x10^{3}$	-	$5.4x10^{3}$	8.7x10
W1	$7.1x10^{4}$	$2.7x10^{3}$	$1.7x10^{2}$	16	abs	$3.7x10^{3}$	-	abs	20
W2	$2.3x10^{4}$	$2.1x10^{4}$	$1.9x10^{2}$	23	abs	$9x10^{3}$	+	abs	24
W3	$2.8x10^{5}$	$6.1x10^{3}$	$4.2x10^{2}$	56	abs	$2.0x10^{3}$	-	abs	$3.0x10^{2}$
W4	$5.2x10^{3}$	$4.6x10^{2}$	61	25	abs	$5.6x10^{2}$	-	1	15
W5	$8.1x10^{3}$	$1.4x10^{2}$	55	22	abs	2.0	-	abs	6
W6	$8.7x10^{4}$	$6.0x10^{2}$	46	19	abs	$2.3x10^{3}$	-	abs	abs
Total /Average	$1.3x10^{7}$	$8.1x10^{6}$	$1.3x10^{7}$	$5.9x10^{5}$	$5.4x10^{2}$	$1.1x10^{4}$		$4.2x10^{3}$	$6.7x10^{2}$

G1-G5 point of wastewater canal, P1-P3 ponds, W1-W6 shallow wells, HPC: heterotrophic counts, TC: total coliforms, FC: fecal coliforms,

In parallel, a **BOD_5** range of canal and pond's wastewater varied from 140 to 36 mg/l with an average 93.2 mg/l. The highest value was obtained in G3; however the lowest values were showed in P3. As we are far from the plant there is decrease in **BOD_5** value, but all groups of canal wastewater exceeded even the maximum value for crops risk according to the FAO value [20, 22-24] which is<25 mg/l. Also this value exceed the result obtained by Akponikpè *et al*. [26]. Similar result was shown with composition of raw wastewater for different parts of the worlds, in USA, France, Morocco (Boujaad), Pakistan (Faisalabad) and Jordan [27]. The BOD_5 level in wastewater is known as an important efficiency indicator of plant treatment. And, the lowest the BOD value, main that is better of quality FAO [28].

Chemical Oxygen Demand (COD): The results obtained with wastewater and Pond's water were varied from 546 to 79 mg/L with an average of 362 mg/L which exceeded 50mg/l of USEPA [23] recommendation and 30- 160mg/l of Feigin *et al*. [29]. Also this was not consistent with reported by AL-Jasser [21] in Saudi Arabia and in Egypt according to the results reported by EL Gammal *et al.* [30].

Turbidity: In this step, samples analysis showed interesting results. The observed values were ranged; respectively, from 440 to 390, 393 to 350 and 0.9 to 0. 40 NTU in canal wastewater, ponds and wells water with an average of 231 NTU. The highest value was obtained in canal wastewater, while the lowest value was found in wells case. This value was exceeded value that reported in literature [1, 21, 23].

NO_3: The results obtained were conforming to Feigin *et al*. [29] recommendation which is <10 mg/l in the studied samples. Results showed a range from 15.9 to 7.3 mg/L with an average of 10.9 mg/L which are similar to that found by Akponikpè *et al*.[26]. It's noticed that nitrite contamination is also often associated with a low bacteriological quality status [31] and NH_4 present in raw sewage would oxidise to NO_3 [32]. Our results relatively to NO_3 content **were within the FAO** recommendation [20].

On the other hand, an Average of **SO_4** content was considerate as normal range because it varied from 145 to 0.4 with an average of 57.3 mg/L. Similar results were found by Al-Jasser [21]. Also, The values of SO_4 were within the standard values (600 mg/l) of wastewater maximum allowable contaminant levels in restricted and unrestricted irrigation waters according to MMRA [33] and MWE [34] recognized in Saudi Arabia.

Microbiological Quality Bienevaluation: Results of analysis of the investigated samples are presented in Table 2. The bacteria counts numerated were high in the most group of canal wastewater and ponds sample as compared to the samples taken from shallow wells. The maximum number value of heterotrophic counts HPC at 22° and 37°C was observed with G4 of $4.2x10^7$, $3.5x10^7$ CFU/ml, respectively, while W4 far away from canal wastewater site had the least microbial load of $5.2x10^3$, $4.6x10^2$ CFU/ml. Accordingly, the HPC for all samples were exceeding the limit of < 1000 CFU/ml EPA [37] ; HPA [35] and [36]. Such result was obtained by Zutter and van [40] when have studied, contamination degree corresponding to total viable bacteria at 37°C and 22 °C, wastewaters from slaughterhouses in Belgium as the incidence level for the range between 10^6 to 10^8CFU/ml.

Results obtained of total coliforms (TC) and fecal coliforms (FC) were in high level with average load values of FC of $5.9x10^5$ CFU/100m. FC of wastewater groups were in average higher than the 5000 CFU/100 ml FAO [28] maximum allowable,1000 CFU/100 ml WHO [37, 38] recommendation concerning wastewater quality reuse to irrigate crops susceptible to be eaten uncooked. Also high level value compared to the MMRA [33] and MWE [34] Saudi Arabia standards for wastewater reuse in agricultural irrigation which not exceed 2.2-1000 per MPN/ 100 mL CFU/100 mL and in Turkey national irrigation water quality standards are equal to 1000 CFU/100 ml [39]. Also this value in contrary with several investigations in different parts of the worlds, in Morocco by Kouraa *et al*. [40], in Aspin total coliform 7300 UFC/100 ml [41], in China actual operating results of the full-scale plant in 1991obtain Total coliform 3-60 (MPN/100 ml) [42], In Saudi Arabia FC ranged between 250 to 25 MPN/ 100 mL [21].

These values indicate high microbial contamination which might explain the inefficiency of Sana'a station in removing bacteria. The main reason to this problem is ascribed to the lack of disinfection units in the plant because station was designed with capacity of 500 **BOD_5** in wastewater per day but it received 1200 **BOD_5** in effluent per day so they didn't add chlorine to avoid form harmful compound. Several reports have confirmed, the relation between the addition of chlorine and of public health protection due to its characteristics of effectiveness [39, 41, 43-45]. Also in time after 12 afternoon when the wastewater treatment plant operating out of its maximum operating load and discharge raw wastewater without treatment specially when it received wastewater with oil or blood hence, it led to contamination of shallow wells [46]. There is an obvious increment between the CFU/100ml. of wastewater canal and shallow wells. Microbial count was higher in W1, W2 and W3 close to wastewater lagoon sit compared to others far away. Various bacteria have ability to growth in a deep subsurface environment [47, 48].

The pond's water shown high level contamination may be return to the water source and surface pollution of cleaned vegetables especially P2 which located in farm 100% use wastewater. This indicates that wash vegetable in it is danger and led to rise of vegetable contamination. Various species of bacteria were adsorbed to vegetable surfaces in wash water [3].

Staphayloccoci spp. counts per 100 ml of canal wastewater and ponds samples have been observed ranged, respectively, from $1.4x10^3$ to $6x10^2$ and $6.7x10^2$ to $3.3x10^2$ CFU. These results agreed with findings reported by Faria *et al.* [49] in Portugal, who mentioned that Staphylococcus prevailed in wastewater treatment plant was estimated to 10^2–10^0 CFU/100 mL. Also, Zutter and van, [50] showed the presence of staphaylococcus with high count in wastewaters from slaughterhouses and was $1.5x10^4$/ml. Many works indicated that staphylococcus was found in water [51-53].

Results obtained concerning *Vibrios spp* ranged from $4.7x10^4$ to $1.3x10^4$ CFU/100ml, in canal wastewater, but there were in case of ponds and shallow wells, respectively, evaluated from $2.0x10^4$ to $3.0x10^2$ and from $9x10^3$ to 2.0 CFU/100ml. These values might explain the poor treatment methods of sterilization. According to this part of work search, the results are compatible to those reported, previously, by Shittu *et al*. [48], Eddabra *et al*. [9], Gopal *et al*. [54] and Miller *et al*. [55]. Survival rates for *V. cholerae* were higher than those reported earlier for ambient waters in Europe. In Italy work by Masinia *et al*. [56] reported that *Vibrio spp* ranged from 10^2 to 10^6 CFU/100ml, also in Spain by Canigrala, *et al*. [57], In South Africa by Igbinosa *et al*. [58] who mentioned that The free-living *Vibrio* densities varied from 0 to $3.45 x10^1$ CFU ml^{-1}, and 0 and $1.9x 10^5$ CFU ml^{-1} in final effluents of a wastewater and in Morocco by Eddabra *et. al*. [9]. Also, *Vibirio* detected in water by other authors Alam *et al*., [59] and Tamrakar *et al*. [60] who detected *Vibiro* in deep ground water. A number of *Vibrio* species other than *Vibrio cholerae* may cause disease in man, mainly by ingestion of contaminated water [58].

Salmonella spp Was found in almost all the wastewater samples. The results oscillated from time to time in the present study. This support the investigated by Shellenbarger *et al.* [61] who isolated eight serovars of Salmonella from slough and pond waters. Such result was obtained in Morocco by Mezrioui *et al*. [62] who isolated *Salmonella spp* from domestic wastewater before and after treatment. The presence of this microorganism has found in analyzed Egypt wastewater [63].

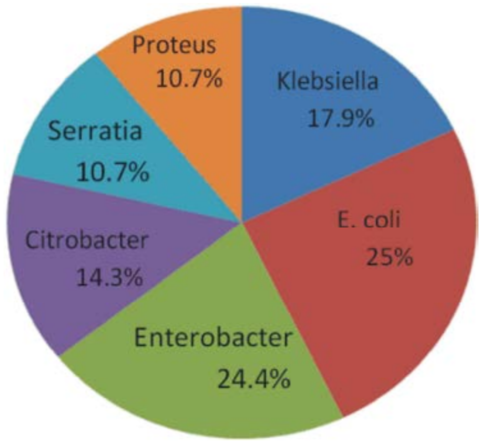

Fig. 1: Identified *Enterobacteriaceae* isolates from the studied water samples

In this work research, because of their importance in food quality, our laboratory team has also aimed isolation of yeasts and molds in tested wastewater samples. Then the results showed presence of yeasts ranged from $4.6x10^4$ to $2.3x10^2$ CFU/ml with an average $4.2x10^3$ CFU/ml. The presence of these fungi has been reported before by Albaum and Masaphy [64] who showed the presence of yeasts in Stream water and Swimming pools with high count. Many species of fungi have ability to grow at rich in organic matter such as the wastewater, sewage, soil which are habitat for many groups of microorganisms [8].

The mean molds was found to be $6.7x10^2$ CFU/ ml the high level was found in P2 $5.3x10^3$ CFU/ml for ponds samples and low level was found in W5 6CFU/ ml of well water sample. Molds in wastewater has been studied in Poland by Kacprzak *et al*. [8], Ulfig *et al*. [65] and in Suadi Aribia by Al-Hazmi [66].

Identification of Enterobacteriaceae Pathogens Isolates: All strains isolated from water samples of different source were Gram negative, short rods and oxidase negative. The identified isolates of Enterobacteriaceaeare presented in percentage (Fig. 1).

Analysis of the isolates revealed that most frequently isolated strains were *Escherichia coli* (25%). *E.coli* in wastewater was an indicator of fecal contamination and it was recorded in previous studies [10, 67]. *E.coli* pollution in water is one of water-borne diseases, which cause many danger infection diarrhea, urinary tract, kidney infections and peritonitis septiceamia [45, 48, 68].

Enterobacter is the scand genus isolated strains which contain three species: *E. aerogenes* (50%), *E. Amnigenus* (33%) and *E. intenrmedius* (17%). Also *Enterobacter* is an indicator of fecal contamination in wastewater. Enterobacter species can be correlated with the presence of pathogenic micro-organisms isolated from wastewater, [69,70]. *E. aerogenes* has been reported to cause bloodstream infections [48, 71].

The *Klebsiella* genus was predominantly represented by *K. pneumoniae* (60%) and *K. oxytoca* (40%). Several studies reported the presence of *Klebsiella pneumonia* in a wide range of water [67, 72, 73]. *Klebsiella pneumonia* was known to be the most dangers species in *Klebsiella* genus; it is the main reason to many problems to human including nosocomial infection, urinary tract infection, diarrhea and other diseases.[74,75].

Serratia was the fifth genus isolated from water samples and was identify by *Serratia marcescens* with percentage (19%). *Serratia* was isolated from water by Yong *et al*. [76] *Serratia marcescens* is generally an opportunistic pathogen causing infections in immunocompromised patients. such as respiratory tract and a lethal septicemia tract infection [77].

Proteus genus was predominantly represented by *Protus microbilis* with percentage (33%) and *Proteus penneri* (70%). *Protus* showed in wastewater by Rehman *et al.* [78]. Also *Proteus mirabilis* was isolated from acclimated sludge from a dyeing wastewater treatment plant [48, 79, 80].

Citrobacter genus was identify by one species *Citerobacter freundii* with percentage (14.3%). *Citrobacter freundii* is a species of *Citrobacter* genus that can be found in water. Many studies has been reported that *Citerobacter freundii* is one of causes of urinary tract infection, [81, 82]. Also *Citrobacter freundii* has been implicated in food spoilage and food poisoning outbreaks [82].

Antimicrobial Susceptibility Profile of Isolates: Isolates antibiotic resistance are presented in Fig. 2. Gentamicin and Cefotaxime recorded 100% sensibility on all isolates. *Klebsiella* isolates showed resistance to Ampicillin recorded the following resistance rates to the used antibiotics: Ampicillin (100%), Amoxicillin (80%), Amoxicillin+Ac clavulanic (20%) and Cefalotin (20%) Ajayi and Egbebi [83] and AlAskari *et al.* [75], who reported resistance of *Klebsiella* to ampicillin, Amoxicillin+Ac clavulanic and Cefalotin. This resistance can constitute a reason and/or difficulty to treat its infection.

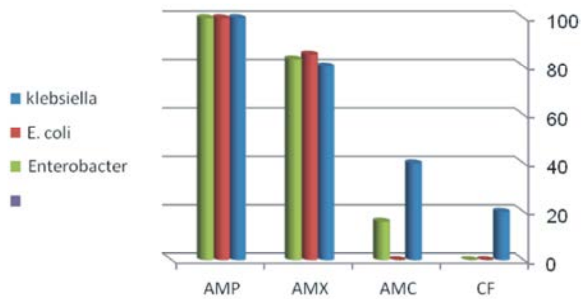

Fig. 2: Antibiotic resistance of some geneses of *Enterobacteriaceae*

Enterobacter isolates have presented resistance to Ampicillin (100%), Amoxicillin (83%), Amoxicillin+Ac clavulanic (16%) and co-trimoxazole (36%). It's noticed that, *Enterobacter* spp. resistance has been reported by Charrel *et al*. [84], who described that *Enterobacter* species are frequently multidrug resistance and their mechanisms can be associated with a change in membrane permeability [85]. While, results obtained with *E. coli* showed resistance in low percentage comparing with other geneses. They presented 100% resistance to Ampicillin which superior to that reported by Alabi *et al.* [68] and Jacoby and Sutton, [86]. And when *E. coli* was tested *versus* Amoxicillin, 85% resistance value was found.

The increasing in recent years of pathogens resistance problem to antibiotics in worldwide, has made public health problems. As per our estimation, health authorities should face this problem in their strategy.

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