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Bacteriological Assessment for Different Water Resources, Ka'am Region, Libya

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التقدير البكتيريولوجي لعينات مياه من مصادر مختلفة بمنطقة كعام بليبيا

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Abstract

Objectives: The principal sources of drinking water in the state of Libya are the desalinated and underground well water. This study was carried out to determine the bacteriological quality of drinking and cultivation water obtained from the stream surface and wells water of the Ka'am Valley in Al-Khoms and the susceptibility of isolated bacteria to antimicrobial agents.

Methods: Water samples taken from 80 different sources were examined for coliform, *Escherichia coli*, and also, Isolated bacteria were tested for their resistance to antibiotics by the BD Phoenix Automated Microbiology.

Results: Of the water samples examined, 3 (3.75%) were positive for *Escherichia coli*, 46 (53.49%) for Coliform spp., and 37 (43.02%) for other gram-negative bacteria. 100% of the bacteria examined were resistant to at least one used antibiotic.

Keywords: *Groundwater, Contamination, Total Coliform (TC), Fecal Coliform (FC).*

الملخص

أجريت هذه الدراسة على 80 عينة مياه بمنطقة كعام الخمس على مدى أربعة فصول متتالية، حيت قسمت العينات إلى 60 عينة مياه أبار و20 عينة مياه سطحية لعين كعام. استهدفت الدراسة تحديد مدي درجة التلوث الميكروبي لعينات المياه وعلاقتها بفصول السنة ومصدرها وقربها من الآبار السوداء وكذلك مدي مقاومة العزلات البكتيرية للمضادات الحيوية. ودلت النتائج المتحصل عليها على عدم صلاحية عدد من مصادر المياه المستخدمة للاستهلاك البشري، نتيجة تعرضها لتلوث بكتيري يعتقد أن مصدره هو مياه الصرف الصحي ومخلفات القمامة والمخلفات الحيوانية. كذلك أظهرت النتائج أن أهم الأنواع البكتيرية القولونية التي تم عزلها خلال هذه الدراسة هي بكتيريا *Escherichia coli* والتي عزلت بنسبة (3.75%) من جميع عينات الدراسة وأيضاً وجدت بكتيريا من أنواع Coliform spp. بنسبة (53.49%)، كما تم عزل العديد من الأنواع البكتيرية المعوية السالبة بنسبة (43.02%)، وأظهرت نتائج اختبارات الحساسية للمضادات الحيوية المستخدمة في الدراسة و التي أجريت على جميع عينات الدراسة مقاومتها للعديد من المضادات الحيوية منها Rifambicin وأمبسلين Ampicilin و Cephalothil في الوقت الذي كانت فيه حساسة لمضادات حيوية أخرى مثل Merobeniem Gentanicin و Amikacine.

الكلمات الدلالية: مياه جوفيه، تلوث، بكتيريا قولونية.

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1. Introduction

Water is a major component of the environment and therefore, is the most indispensable natural resource which is essential for life and health on earth. The World Health Organization (WHO) attributed 4.0% of all deaths and 5.7% of the global disease burden to water-related illnesses, which stemmed from poor water quality, hygiene and sanitation (Pruss *et al*., 2002). Groundwater is the major source of water to the Libyan people includes Ka'am region used for different purposes. Majority of the population in the residential area depend on wells as their source of water supply. There is no information available regarding the population's water quality before.

However, Libyan countries is arid and semi-arid regions have depended heavily on the groundwater. Water derived from the traditional sources (wells), showed increases in most of the investigated bacteriological parameters, followed by surface water as compared to bottled or desalinated water. The wells and surface water are at risk of contamination as indicated by the higher levels of most bacteriological parameters. Nonetheless, groundwater is still and will continue to be the main safe source, reliable drinking water, and matter of serious concern today especially in countryside areas as Ka'am region.

Contaminants can find their way into drinking water sources through microorganisms from human or animal excreta, surface runoff, leakage of microbial landfills septic reservoir effluents and indiscriminate dumping of wastes in streams or directs to the wells (Gasana *et al*., 2002; and Al-Khatib *et al*., 2003). Coliform bacteria are used for monitoring the bacteriological safety of water supplies on the basis of the realization that the presence of coliform bacteria or fecal bacteria in water is an indicator of possible human fecal contamination, and therefore the likely presence of enteric pathogens. The presence of coliform bacteria in drinking water indicates that other disease-causing organisms (pathogens) may be present in the water source or its distribution system. However enteric pathogens include members of the genera Escherichia, Enterobacter, Citrobacter, and Klebsiella among other bacteria. Wherever, *E. coli* is primarily associated with human feces, it is a useful pointer of human fecal contamination of water and the appropriate focus of monitoring for indicators of potential enteric pathogens in either ground or surface waters (Tallon *et al*., 2005; and Odonkor and Ampofo, 2013).

Drinking water contaminated with *E. coli* is known to cause stomach and intestinal illness including diarrhea, jaundice, typhoid, nausea, and other problems. (Gwimbi, 2011). The public health implications of drug-resistant and high proportions of antibiotic resistance in bacteria that cause common infections, and emergence of bacteria resistant to antibiotics is common in areas where antibiotics are used. Bacteria have developed different mechanisms to render ineffective the antibiotics used against them, these antibiotic-resistant bacteria are increasingly occurring in contaminated areas, such as intensive animal husbandry which causes resistant bacteria to enter the environment directly from liquid manure and muck

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(Aarestrup *et al*.,1996). The other polluted factors could transport to the water (Ashbolt, 2004; Prasai *et al*., 2004.; and Clark and Pagel, 1977).

The present study was carried out to monitor the suitability of ground and surface water for safe drinking purposes and to investigate the status of these sites for bacterial contamination throughout the study period. This study was undertaken to accomplish two goals; the first, to determine the bacteriological quality of drinking and cultivation water obtained from the stream surface water of the Ka'am valley (Alain) and water wells, the second goal was to provide detailed descriptive information about the antibiotic resistances of gram-negative bacteria isolated from different water sources.

2. Material and Methods

2.1. Study Area

The study was conducted in Ka'am region in AlKhoms city, it has a population of 28,000 inhabitants. Majority of the people of housing colonies use ground water for domestic purpose. The town located within the North Western, part of the Libya, the town lies between latitude of N 32.29' – 32.34' and longitude of E, 14.21' – 14.28', temperature ranges from 0-15°*C* in Winter night to 15-25°*C* at day, where in Summer day between 30- 45°*C*, and 15-25°*C* at night (MFF, 2015). Figure (1) present a location map of the studied area.

Figure 1. Location map of the studied area.

2.2. Collection of Water Samples

Water samples from eighty sources were randomly collected for bacteriological analysis, during four seasons in 2014, each season 20 samples were collected included 5 samples from

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Alain surface directly. Samples were collected aseptically in sterile glass containers (100 *ml*) containing 0.1 *ml* sodium thiosulphate (1.8% *w/v*) to neutralize the bacterial effect of chlorine or chloramines in the water. The bottle cap was aseptically removed and the weighted bottle lowered into the to a depth of about 15-20 *cm*, the bottle was brought up to a surface and covered with a screw cap, no air bubbles were seen inside, 500 *m* were pointed as a space from each sample. Wells depth ranges from 18-85 *m*, all collected samples from wells were allow to raining 5 *min*. before collected. Samples were transported to the laboratory in a cool container within two hours (Nogueira *et al*., 2003).

2.3. Bacterial Isolation and Identification

Standard bacteriological techniques were used to detected total coliforms (TC) and faecal coliforms (FC). In other words, the five tube most probable number technique (MPN) was employed for the total bacteria (FC, and TC) in Autumn and Winter time (Sleigh and Duguid, 1989), whereas in Spring and Summer, the dry plate count agar was applied in time (Reasoner and Geldeich, 1985). For isolation of coliforms and faecal coliforms a loopful from each tube positive for *E. coli* and coliform spp. was plated onto MacConkey agar plate and incubated at 37^oC overnight. Isolated bacteria were identified by standard bacteriological techniques (APHA, 1998). Undefined isolates were identified by other method by using Phoenix Automated Microbiology System (Biosciences, Sparks, MD, USA) (CLSI, 2008). Furthermore, antibiotic sensitivity testing methods were done to isolated bacteria (Senior, 1989).

3. Results

Our study, showed that 18 bacterial species were isolated from 80 different water samples collected during the four seasons. These bacterial species included 86 bacterial isolates. TC and FC counts ranged between 0->$1.6x10^3$/*ml* and 2-7.8/*ml* respectively. Of the total samples examined, *E. coli* was detected in three samples (3.75%), *Yersinia pseudotuberculosis* in 12 samples (15%) and *Klebsiella pneumonia*e in 12 samples (15%), whereas *Hafnia alvei* and *Citrobacter freundil* in five sample (6.25%) for both and Coliform spp. in 9 samples (11.25%). The difference in the isolation rates of *E. coli* during the four seasons was not statistically significant ($P>0.05$). Whereas *Yersinia pseudotuberculosis* was significantly more identified from water samples in Autumn (37.1% (23/62)) than from water samples in Winter ($P<0.006$) and more than Spring and Summer ($P<0.003$). Coliform spp. was significantly detected in Autumn compared with Coliform spp. isolated in Winter and Summer ($P<0.002$), whereas in Spring ($P<0.009$). *Klebsiella pneumonia*e found in high number in Autumn than in Spring and Summer ($P<0.004$). Frequency of bacterial agents isolated from 80 water samples and their relation to seasonal variation during the study are shown in Table (1).

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Table 1. Frequency of bacterial agents isolated from 80 water samples and their relation to seasonal variation during the study

Isolation N(%)					
Bacteria spp.	***Seasons***				
	Summer N=20	***Spring N=20***	***Winter N=20***	***Autumn N=20***	***Total N=80***
One spp.	9(45)	10(50)	6(30)	6(30)	31(38.75)
More than to one spp.	0(0.0)	0(0.0)	10(50)	11(55)	21(26.25)
Yersinia pseudotuberculosis	0(0.0)	0(0.0)	2 (10)	10(50)[1]	12(15)
Klebsiella pneumoniae	0(0.0)	0(0.0)	5(25)[3]	7(35)[2]	12(15)
Klebsiella rhinoscleromatis	2(10)	7(35)[4]	2(10)	0(0.0)	11(13.75)
Coliform spp.	0(0.0)	1(5)	0(0.0)	8(40)[5]	9(11.25)
Citrobacter freundii	2(10)	0(0.0)	3(15)	0(0.0)	5(6.25)
Hafnia alvei	0(0.0)	0(0.0)	3(15)	2(10)	5(6.25)
Yersinia spp.	0(0.0)	0(0.0)	5(25)[6]	0(0.0)	5(6.25)
Salmonella spp.	1(5)	0(0.0)	1(5)	2(10)	4(5)
Edwardsiella tarda	0(0.0)	0(0.0)	3(15)	1(5)	4(5)
Shewanella putrefaciens	4(20)[7]	0(0.0)	0(0.0)	0(0.0)	4(5)
Escherichia coli	0(0.0)	1(5)	0(0.0)	2(10)	3(3.75)
Proteus mirabilis	0(0.0)	0(0.0)	0(0.0)	3(15)	3(3.75)
Citrobacter eintermedius biotype	0(0.0)	0(0.0)	2(10)	1(5)	3(3.75)
Enterobacter aeragenes	0(0.0)	1(5)	0(0.0)	1(5)	2(2.5)
Serratia marcescens	0(0.0)	0(0.0)	0(0.0)	1(5)	1(1.25)
Enterobacter cloacae	0(0.0)	0(0.0)	1(5%)	0(0.0)	1(1.25)
Shigella sonnei	0(0.0)	0(0.0)	1(5)	0(0.0)	1(1.25)
Yersinia enterocolitica	0(0.0)	0(0.0)	1(5)	0(0.0)	1(1.25)

1) *Yersinia pseudotuberculosis* was significantly isolated in Autumn than compared with Winter and Summer (P<0.006 and P<0.003 respectively). ***2)*** *Klebsiella pneumonia* was isolated in Autumn and not reqnized in either Spring or Summer with (P<0.004). ***3)*** *Klebsiella pneumonia* was more isolated in Winter than Spring and Summer (P<0.02). ***4)*** *Klebsiella rhinoscleromatis* found hgiher in Spring than in Winter and Summer season (P<0.005) (OR=4.85), even higher than Autumn (P<0.004). ***5)*** Coliform spp. were found to be higher in Autumn than in Winter and Summer (P<0.002), where also higer than Speing season (P<0.009). ***6)*** Yersinia spp. was found higher in Winter compared with the other seasons (P<0.002). 7) *Shewanella putrefaciens* was isolated in Summer only (P<0.004).

According to type of water sources Table (2), most of bacterial species were not found to be significantly more isolation from any season than another (P>0.05). Salmonella spp. was found to be significantly more common in Alain than in wells which isolated from 3 (15%)

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and one (1.66%) sample from Alain and wells respectively (P<0.02). Of 77 isolated gramnegative pathogenic bacteria (3 *E. coli*, 40 coliform spp. and 34 another gram negative spp.) 89.61% were resistant to Erthromycin, 88.31% to Rifampicin, 85.71% to Ampicillin, 81.82% to Amoxicillin. *E. coli* isolates were susceptible to Amoxicillin, Chloromphenicol, Nitrofurantion, Cephalothin and Colistin. Antimicrobial resistance profiles of bacterial strains isolated from water shown in Table (3).

4. Discussion

WHO estimates that 80 % of all sickness in the world can be attributable to inadequate potable water supplies and poor sanitation (Pant, 2004). This is the first study as far as we know in the region, to assess the quality of water resources and prevalence of the resistance isolated bacteria on the Ka'am region.

Escherichia coli was one of the most isolated important fecal indicators, where isolated from (3.75%) during the study, this result is in agreement with Algobaar (2008), who found that *E. coli* isolated from Aryad drinking wells (4.93%), also EL-Jakee (2009) found that *E. coli* 7.7% among water samples collected from drinking underground, other study have been reported higher percentage than this finding. *E. coli* was isolated from different water samples (37.3% and 51.51%), respectively (Aboagallah, 2013; and Ali *et al*., 2014). Detection of *E. coli* indicates direct or indirect contamination of water by animal or human feces, which in turn means the possible presence of serious enteric pathogens that include among others klebsiella spp., diarrheagenic *E. coli*, and enteric viruses in such water sources.

Other microorganism, *Shewanella spp*. was isolated from the same sources 4(5%) in which; one sample from Alain 1/20 (5%) and 3/60 (3.33%) from the wells, whereas Shewanella *spp*. is a marine organism, other investigators reported relatively similar findings, Koziñska and Pekala (2004), who isolated the bacteria from fish in fresh water. *Klebsiella pneumonia*; *Yersinia pseudotuberculosi*s, isolated at 15% from water samples followed by *Klebsiella rhinoscleromati*s and coliform ssp.11(13.75); 9(11.25) receptively, these results confirmed that animals or human feces are a main source of contamination (Chiesa *et al*., 1993; and Podschun *et al*., 2001), especially if we take into our consideration that this area is used as cattle market, grazing of livestock and also pens for the cattle's, where manure is used as a common soil fertilizer.

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Table 2. Distribution of bacterial isolated according to the source of water and the space near the sewage sources.

Isolation N(%)						
Bacterial spp.	***Water source***			***Near the wells from sewage***		
	Alain N=20	***Well N=60***	***P.V***	***Yeas N= 52***	***NO N= 8***	***P.V***
	4(20)	8(13.33)	NS	8(15.38)	0(0.0)	NS
	2(10)	10(16.66)	NS	10(19.23)	(0.0)0	NS
	2(10)	9(15)	NS	9(17.30)	(0.0)0	NS
Yersinia pseudotuberculosis	2(10)	7(11.66)	NS	7(13.46)	(0.0)0	NS
Klebsiella pneumoniae	3(15)	2(3.33)	NS	2(3.84)	(0.0)0	NS
Klebsiella rhinoscleromatis	2(10)	3(5)	NS	3(5.76)	(0.0)0	NS
Coliform spp.	2(10)	3(5)	NS	3(5.76)	(0.0)0	NS
Citrobacter freundii	3(15)	1(1.66)	0.02	(0.0)0	1(12.5)	0.02
Hafnia alvei	1(5)	3(5)	NS	3(5.76)	(0.0)0	NS
Yersinia spp.	1(5)	3(5)	NS	2(3.84)	(0.0)0	NS
Salmonella spp.	2(10)	1(1.66)	NS	(0.0)0	1(12.5)	0.02
Edwardsiella tarda	2(10)	1(1.66)	NS	1(1.92)	(0.0)0	NS
Shewanella putrefaciens	1(5)	3(3.33)	NS	2(3.84)	(0.0)0	NS
Escherichia coli	1(5)	1(1.66)	NS	1(1.92)	(0.0)0	NS
Proteus mirabilis	1(5)	0(0.0)	NS	0(0.0)	(0.0)0	NS
Citrobacter eintermedius biotype	0(0.0)	1(1.66)	NS	1(1.92)	(0.0)0	NS
Enterobacter aeragenes	1(5)	0(0.0)	NS	(0.0)0	(0.0)0	NS
Serratia marcescens	(0.0)0	1(1.66)	NS	1(1.92)	(0.0)0	NS
Enterobacter cloacae	4(20)	8(13.33)	NS	8(15.38)	0(0.0)	NS
Shigella sonnei	2(10)	10(16.66)	NS	10(19.23)	(0.0)0	NS
Yersinia enterocolitica	2(10)	9(15)	NS	9(17.30)	(0.0)0	NS

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Table 3. Antimicrobial resistance of bacterial strains isolated from 80 waters samples

No (%) Resistance				
Antibiotics	***Escherichia coli N=3***	***Coliform spp[1] N=40***	***Gram negative Spp. N=34***	***Total N=(77*)***
Chloramphenicol(C)	0(0.0)	19(47.5)	15(44.12)	34(44.16)
Gentamicin(CN)	1(33.3)	8(20)	8(23.52)	17(22.10)
Meropenem(MEM)	1(33.3)	11(27.5)	5(14.71)	16(20.78)
Amikacin(AK)	0(0.0)	8(20)	6(17.65)	14(18.18)
Ampicillin (AMP)	1(33.3)	7(92.5)	29(85.29)	66(85.71)
Erythromycin(E)	1(33.3)	8(95)	31(91.18)	69(89.61)
Ceftazidime(CAZ)	1(33.3)	10(25)	2(5.88)	12(15.58)
Rifampicin(RD)	2(66.6)	37(92.5)	31(91.18)	68(88.31)
Nitrofurantion(F)	0(0.0)	25(62.5)	15(44.12)	40(51.94)
Cephalothin(KF)	0(0.0)	34(85)	22(64.71)	56(72.73)
Aztreonam(ATM)	2(66.6)	18(45)	6(17.65)	24(31.16)
Amoxicillin(AML)	0(0.0)	36(90)	27(79.41)	63(81.82)
Cefuroxime(CXM)	2(66.6)	27(67.5)	23(67.65)	50(64.93)
Colistin(CT)	0(0.0)	9(22.5)	10(29.41)	19(24.68)

*77 isolates were tested of total isolated bacteria (86 isolates)

Coliform spp. Includes *(Klebsiella pneumonia; Klebsiella rhinoscleromatis; Citrobacter intermedius biotype; Citrobacr freundii; Enterobacter clocae* and *Hafnia alvei*).
Gram negative includes all the other species except coliform spp.

Another influence to be considered in the survival rates are the seasonal variation, it well known that fecal survival bacterial rates can vary from a few minutes to many days depending upon the environmental conditions (Hughes, 2003). In this study seasonal variations of bacterial populations and their occupancy were surveyed in the water sources, and studies have shown that the numbers of bacteria increase in their densities (Drasar *et al*., 1981; Tripathi and Sharma, 2011; Javed *et al*., 2014; Nogueira *et al*., 2003; Maipa *et al*., 2001; and Van Donsel *et al*.,1967). In a study nearby the area have reported that the coliform bacteria were highly isolated in Autumn and Winter (Almhgoob, 2005). Furthermore, a high percentage (26.25%) of multiple agents were more prevalent in Autumn and Winter, while the same bacteria not cultured in Spring and Summer. However, this study reported that there is no clear differences found between number of isolated bacteria from either wells closed or faraway from sewage wells. *E. coli* was not isolate from 52 wells closed to sewage wells, on

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the other hand *E. coli* was isolated from a well faraway from sewage sources but close to the livestock and birds cages. On the basis of the above discussion, it may be concluded that the underground drinking water at almost all the sites at Ka'am was highly polluted as indicated by either animal waste or human activity.

Presence of pathogenic bacteria that resistant to the drug potentially may play a role in the spread of multidrug-resistant bacteria in the community and pose a serious health risk to society (Schwartz *et al*., 2003). Many studies have been demonstrating that a significant increases of multiple-antibiotic resistant bacteria occur in various drinking water systems (e.g. Calomiris *et al*., 1984; and Ali *et al*., 2014), however this study have spotted that 80% of all examined bacterial strains were resistant to Ampicillin, Amoxicillin and Rifampicin. Therefore, effort should be made by relevant authorities to conduct quality assessment of properly use of antibiotics, and monitoring water sources from time to time in order to ensure that safe drinking water.

5. Conclusion

The detection of total coliforms and *E. coli* in some of the water samples from different water sources in Ka'am and even resistances bacteria implies that the contaminated drinking water may be due to exposure wells and surface water to microbiological contamination either by human activities or the presence of animals.

Finally, we hope that this study may encourage other investigators from this or other regions to carry out more studies on the bacteriological quality of water used for drinking and other purposes provided by houses, farms and public utilities of different regions, which in turn may provide a better idea on the quality of such water.

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