

Detection of Three uncommon Bacteria in Tigris River and Purification Stations

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Abstract:

During the evaluation of water quality before and after treatment in one of the three water purification stations in Baghdad city.

Three uncommon bacterial species were detected and isolated from the water of Tigris River and the drinking water after treatment in purification station using conventional bacteriological methods and API_{20E} technique for the identification of bacteria.

The isolated bacterial species were *Pantoea* spp., *Rahnella aquatilis* and *Sphingomonas paucimobilis* which were belonging to the families Enterobacteriaceae and Sphingomonadaceae.

It is of noteworthy that, these species has not been previously isolated from the water of Tigris River or from any other Iraqi environment or clinical samples.

Although, these bacteria were habituated natural environment (water, plants and soil), they are opportunistic pathogen and they sometime pose a real public health concern, they could infect different parts of human body and some of them were recovered from different hospital environments and equipments.

الخلاصة :

من خلال تقويم المياه من الناحية البكتريولوجية قبل و بعد عمليات التصفية في بعض محطات معالجة المياه في بغداد, عزلت ثلاثة انواع من البكتيريا غير الشائعة من مياه دجلة ومياه الشرب بعد التعقيم في محطة التصفية. هذه الانواع من البكتيريا هي, *Pantoea* spp., و *Rahnella aquatilis* و *Sphingomonas paucimobilis* لقد استخدم في تشخيص هذه الانواع من البكتيريا الطرق البكتريولوجية المعتادة و كذلك عدة ال API_{20E} الخاصة بتشخيص البكتيريا. شخصت بكتيريا *Pantoea* spp., و *Rahnella aquatilis* ضمن عائلة البكتيريا المعوية اما بكتيريا *Sphingomonas paucimobilis* فقد شخصت ضمن عائلة Sphingomonadaceae. ومن الجدير بالذكر ان هذه الانواع من البكتيريا لم تعزل سابقا من البيئة العراقية لا من مياه نهر دجلة و لا من العينات البيئية او السريرية الاخرى. وبالرغم من ان هذه الانواع من البكتيريا تعيش في مختلف البيئات الطبيعية من مياه و نباتات و تربة, الا انها قد تكون انتهازية مرضية احيانا و قد تؤدي الى مخاطر صحية تصيب افراد المجتمع. ان هذه البكتيريا قد تصيب الانسان بامراض خطيرة مختلفة, ويمكن عزلها ايضا من عينات سريرية وغير سريرية مختلفة من المستشفيات.

Introduction:

Water is essential for industrial, pharmaceutical and hospital purposes, it is specially used for cleaning and hygiene purposes, and the greatest demand on water is destined for human consumption, therefore its quality being relatively guaranteed up to the point where the pipe transportation network terminates^[1].

Human being, on the other hand, will continue to pollute the water reserves with different pollutant, bioaccumulation,

agricultural, industrial radioactive, and most important is the contamination with microorganisms, especially those of the member Enterobacteriaceae, *E. coli*, *Shigella* spp., *Proteus* spp., *Yersinia* spp., *Salmonella* spp. and others^[2].

Water for human consumption is usually disinfected before being distributed to the consumers to ensure that the level of any potential harmful bacterial species fall under defined low level, for example, zero fecal coliform per 100 ml. In many instances, the quality of the water may have

deteriorated by the time it reaches to the consumers.

This is often due to the recontamination after treatment owing to the regrowth of sublethally damaged bacteria harbored in biofilms^[3]. According to the WHO^[4], drinking water should be free from any organism that might pose a health risk to the human population.

During the evaluation of water quality of Tigris river, the microbial analyses of Tigris river water were performed before and after purification in the water treatment system unit including estimation of the number of total viable microbial counts, total viable *E. coli* and *Pseudomonas aeruginosa* and other pathogenic enteric microorganisms e.g. *Salmonella* spp. and *Vibrio cholera*.

Three rare pathogenic bacterial species, *Pantoea* spp. *Rahnella aquatilis* and *Sphingomonas paucimobilis* were detected for the first time in Iraqi environment. These bacteria were widely distributed in soil, water, plant surface and human body^[5]. However, many researchers reported that *Sphingomonas* spp. are a causative agents of human infection^[5,6]. *Pantoea* spp. involved in oncology patient, neonatal ICU and of worldwide epidemic of septicemia^[7], osteitis^[8], cholelithiasis^[9], occupational respiratory infection and skin allergy^[10], blood stream infection in an elderly person^[11], and peritonitis^[12]. While *Rahnella aquatilis* was isolated from blood culture of oncology patient and patients from ICU department^[7].

In Iraq, there are no any reports dealing with the detection and isolation of any of these bacteria from various Iraqi environmental communities or from human or plant bodies in spite of the importance of these microorganisms in nature and to the health of man. Therefore, the main objective of this research is initially to isolate these bacteria from Tigris river water and from the water used for human consumption.

Materials and Methods:

1- Sample collection:

Water samples from Tigris river (20-30cm under the surface of water from the point where it inters to the pipe of purification station) and from the purification station faucets (after sterilization of the opening of the faucets with alcoholic burner and to let out water for two minutes) were collected during 2010 in 250 ml. sterile bottles containing sterile sodium thio-sulphate to a final concentration 0.01% (w/v) to neutralize any free or combined residual chlorine. All the samples were transported to the laboratory on ice and analyzed within 12h.

The purification stations were include Al-Karama station, located at the center of Baghdad, and provides parts of Al-Rusafa and part of Al-Karkh sides of Baghdad with drinking water, and sharq Diglla station located at north of Baghdad and it provides Al-Rusafa side of Baghdad with drinking water and Al-Qadissiya station, located at the south of Baghdad and it provides Al-Qadissiya region and around with drinking water^[13].

2- Filtration and culturing of water samples:

Water from Tigris river and from the purification station were tested for the presence of total coliform by filtering duplicate 100ml. volume through 0.45 μ m nitrocellulose filter (Millipore) and incubated on m Endo agar at 35⁰C for 24h. For heterotrophic bacterial counts the Millipore filters were incubated on Tryptic Soya Agar (TSA), Difco, Detroit, Michigan (USA) at 37⁰C for 72 h.

3- Isolation and Identification of colonies:

After 24hr incubation, cultures were examined for distinct colonies, the colonies were transferred on to surface of nutrient agar in plates and incubated at 37⁰C for 18-24 h. All the colonies were tested for colony morphology and colony color. The identification tests for genera and species

of each isolate were performed using conventional bacteriological methods^[14], and confirmed by using API_{20E} kit (Bio-merieux) France .

Results and Discussion:

The enumeration of different potential pathogenic bacteria from water of Tigris and purification station revealed the isolation of a total of (103) isolates from Tigris river water and (46) isolates from purification station with different aspects, circular, sharp brilliant, opaque, light brown, dark brown, pink, orange, white and cream colored colonies. All these colonies were confirmed to indicate the presence of at least (13) different species of bacteria belonging to the families Enterobacteriaceae and Sphingomonadaceae.

According to the API_{20E} kit used in the identification of Gram(-ve) lactose fermenting and lactose non-fermenter bacteria, the total number of colonies found in water of Tigris river and purification station (CFU/ 100 ml.) and picked for identification are shown in Table 1. There was a prevalence of isolation of *Pseudomonas* spp. and *E. coli* in both Tigris River and in the station after purification and to a lesser percentages for the rest bacterial isolates.

Along with these potential pathogens, rare species of bacteria were also isolated, these were , *Pantoea* spp, *Rahnella aquatilis* and *Sphingomonas paucimobilis* ,these bacteria has not been previously isolated from water samples of Tigris or from any other environmental or clinical sources in Iraq.

Pantoea spp. and *Rahnella aquatilis* are Gram (-ve), lactose fermenters produce yellow colonies while *Rahnella aquatilis* form white or opaque entire edges colonies. Based on API_{20E} test, both species were urease negative and lysine and ornithine were not carboxylated, they uses glucose,

arabinose, mannitol, rhamnose, and sucrose as substrate for energy production, and Vogus-Proskaure are positive^[15]. *Sphingomonas paucimobilis*, on the other hand, form a yellow colored colonies of entire edge and are urease negative, do not reduce nitrate, it is positive for both catalyse and oxidase, lysine and ornithine are not decarboxylated and it utilizes glucose, sucrose and maltose for energy production and lactose not fermented^[16].

Pantoea spp. , *Rahnella aquatilis* and *Sphingomonas paucimobilis* are considered opportunistic pathogens and very common in nature, soil, water, plants (including fruits and vegetable), animals and organic material decomposition (sewage). In the present study, they were found in water treatment system, demonstrating an adaptation to environment with low concentration of nutrients and a large range of temperature from 4°C to 42°C.

Pantoea spp. was reported to be a causative agents of sever diseases of man and was isolated from different parts of the body, from children, new born babies, blood, joints, urinary tract infection and medical instruments, as well as from forest trees around the world and even from tanks of oil of jet air-plan^[7]. *Rahnella aquatilis*, on the other hand, usually found in fresh water and isolated from bronchial washing of patient with acquired immunodeficiency syndrome (AIDS)^[17] and from sickle cell diseases^[18].

Although infection by *Sphingomonas paucimobilis* are rarely serious and could be effectively treated with antibiotics, it can cause active infection in human, e.g. bacteremia, peritonitis, brain abscess, respiratory and urinary infections, meningitis etc. and also recovered from hospital environment including tap water distilled water, nebulizers, dialysis fluids and other equipments^[19,20]. And it could utilizes a wide range of organic compounds, toxic materials and some types of environmental contaminants, therefore, studies have been

held to further explore its metabolic mechanisms for more applications in Biotechnology^[21].

Finally, it is recommended that a further studies should be done and focusing

on these species of bacteria, and their rule in producing diseases in Iraqi people, and trying to solve problems of such diseases of unknown causative agents .

Table -1: Total number and percentages of identified species of Gram (-ve) bacteria isolated from the waters of Tigris River and from the purification station.

Bacterial species	No. and % of species isolated from			
	Tigris river		Stations	
	No. of isolates	%	No. of isolates	%
<i>Pseudomonas spp.</i>	33	32.03	18	39.13
<i>E.coli</i>	30	29.12	10	21.73
<i>Serratia spp.</i>	1	0.97	1	2.17
<i>klebsiella spp.</i>	15	14.56	4	8.69
<i>Citrobacter spp.</i>	5	4.85	2	4.34
<i>Enterobacter spp.</i>	5	4.85	4	8.69
<i>Proteus spp.</i>	1	0.97	0	0
<i>Aeromonas spp.</i>	2	1.94	1	2.17
<i>Vibrio spp.</i>	6	5.82	0	0
<i>Salmonella spp.</i>	0	0	1	2.17
<i>Sphingomonas Paucimobilis</i>	1	0.97	2	4.34
<i>Pantoea spp.</i>	2	1.94	2	4.34
<i>Rahnella aquatilis</i>	2	1.94	1	2.17
Total	103	99.96	46	99.94

References:

- 1- Penna, T. C.; Silva, A. M. M. and Priscila G. M. Identification of bacteria in drinking and purified water during the monitoring of a typical water purification system. BMC Public Health 2002. vol.2
- 2- Macfeters, G. A. Drinking water microbiology. Progress and recent development. Spring. Newyork. 1990.
- 3- Lee, D.G. and Kim. S. J. Bacterial species in bio-film cultivated from the end of soul water distribution system. J. Appl. Microbiol. 2003. Vol. 95.Pp: 317-324 .
- 4- WHO Guidelines for drinking water quality, 3rd edition. WHO, Geneva. 2004.
- 5- Nishiyama, N.; Sanoo, K.; Wada, H. and Matsumoto, S. Identification of soil microhabitate for growth, death and survival of bacteria, g-1, 2, 3, 4, 5, 6-hexachlorocyclohexan assimilating *Sphingomonas paucimobilis* by fractionation of soil .FEM Microbiol. Ecol. 1992. Vol . 101. Pp: 145-150.
- 6- Winn, W. C.; Allen, S. D. ; Janta, W. M.; Koneman, E. W. and Procop, G. W. Schreckenberger, P. C, et alKoneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th edition Philadelphia: Lippincott Williams and Wilkins. 2006.

- 7- Habsah, H.; Zeehaida, M.; Van Ros, L H.; Noraida. R.; Wan Pauzi, W. I.; Fatimah, I.; Rosliza, A. R.; Nik, N. Y. and Maiman, H. An outbreak of *Pantoea* spp. in a neonatal Intensive Care Unit secondary contaminated parental nutrition. J. Hosp. 2005. Inf. Vol. 61. Pp: 213-218.
- 8- Luporte, C.; Demachy, M. C. and Thevenin-Lemoine, C. Tibial osteitis caused by *Pantoea agglumerans* after open grade III B tibial Shaft fracture. Rev. Chir. Orthop. Reparatrice Appar. Mor. 2002.Vol. 88. Pp: 625-627
- 9- Flores, C.; Maguilnik, I.; Hadlich, E. and Goldani, L. Z. Microbiology of choledochal bile in patients with choledcholithiasis admitted to a tertiary hospital. J. Gastroenterol. Hepatol. 2003. Vol. 18. Pp: 333- 336.
- 10- Milanowski, J. D.; Potoczna, H.; Kuz, L. and Urbanpnicz, B. Allergic alveolitis among agricultural workers in eastern Poland: A study of twenty cases. Ann. Agric. Environ. Med. 1998. Vol. 5. Pp: 31-34.
- 11- Baaere, D. E.; Verheslst, R.; Labit, Verschraegen, G.; Wauter, G.; Clayes ,G. and Veneechoutte, M. Bacteremia infection with *Pantoea ananatis*. J. Clin. Microbiol. 2004. Vol. 42. Pp: 4393-4395.
- 12- Lim, P. S.; Chem, S. L.; Tsai, C.Y. and Pai, M. A. *Pantoea peritonitis* in a patient receiving chronic ambulatory peritoneal dialysis. Nephrology (Carlton). 2006. Vol.11.Pp: 97-99.
- 13- Al-Bayatti Khalid, K.; Al-Araji Kadhun, H. and Al-Nuaimy Seba H. Bacteriological and Ecological studies on Tigris River near the water purification stations within Baghdad province.(under publication) 2012 .
- 14- The Microbiology of water, Part 1- Drinking water Report on Public Health and Medical subjects, No.71. Methods for the examination of water and Associated Methods. London, HMSO 1995.
- 15- Alexis, D.; Dominique, D.; Stephane, C.; Virgine, P.; Jennifer, A.; Patrick, G.; Guillaume, A. and Sylvain, B. Phylogeny and Identification of *Pantoea* spp. and Typing of *Pantoea agglumerans* strain by Multilocus gene sequencing J. Clin. Microbiol. 2009. Pp: 300-310 .
- 16- Masahiro, K.; Nozo, Mi. I. and Toshiaki, K. Biological pre-treatment with two strains for enzymatic hydrolysin of office paper, Current Microbiol. 2007. Vol. 54. Pp: 424-428.
- 17- Harrel, L .g.; Cameron, M. L. and Ohara, C. M. *Rahnella aquatilis* an unusual G(-) rod isolated from the bronchial washing of a patient with acquired immunodeficiency syndrome. J.Clin. Microbiol. 1989. Vol.27 (7). Pp: 1671-1672
- 18- Gaitan, J. I. and Bronze M. S. Infection caused by *Rahnella aquatilis*. Am. J of Med. Sci. 2010. Vol. 339 (6). Pp: 577-579.
- 19- Hsueh, R. R.; Teng, L. T.; Yang, P. C.; Chen. Y. C.; Pan, H. J. and Ho, Sw. Nosocommial infections caused by *Sphingomonas paucimobilis*: Clinical features and microbiological characteristics. Clin. Infec. Dis. 1998. Vol. 26 Pp: 676-681.
- 20- Abdullah, K.; Zaynep, S. A.; Emink, H. A, Kenan, S.; Erol, K. and Celal, B. Nosocommial outbreak of *Sphingomonas paucimobilis* bacteremia in a Heamato/oncology unit. Jpn. J. Infect. Dis. 2007.Vol. 60 Pp: 394-396.
- 21- Nilgiriwala, K. S.; Alahari,A.; Rac, S. A. and Apte, S. k.Cloning and over expressing of alkaline phosphatases phoR from *Sphingomonas* spp. strain BSAR-1 for Bioprecipitation of Uranium from Alkaline Solutions. Applied and Environmental Microbiology. 2008. Vol. 74 (17) Pp: 5516-5523.