

## **Studies on *pseudomonas aeruginosa* in drinking water from water suppliers consumed in Basra.**

**Ghazi M. Al-Maliki & Rajaa Abdul-Kadem Hanef**

*Department of Marine Biology, Marine Science Center, University of Basrah, Iraq.*

### **Abstract**

Forty samples of drinking water collected from water supplier's consumed in different (10) regions in Basra city through six months at 2012, have been analyzed bacteriologically. Pseudomonads members were selectively investigated and identified biochemically and showed as (14 %). The majority of *Pseudomonas* spp. were found to be resistant to nalidixic acid, tetracyclin, ampicillin, and ceftazidim. The most effective antibiotics against *Pseudomonas* spp. were ciprofloxacin, gentamicin, colistin, and ceftriaxone. The high load of opportunistic pathogen such as Pseudomonads in this study represents hygienic and quality challenge for consumed water, multiple antibiotic resistance detected among these bacteria might pose health significances, at least to some defined sensitive individuals, and should be considered properly.

### **Introduction**

There has been a dramatic increase in the consumption of drinking water, especially in Basrah. Objection to the local water supplies for offensive taste and odor as well as fluoride, chlorine and other additives might be considered as additional factors for water pollution (31; 29; 2). Because the emergent supplied water is an oligotrophic environment, their content of viable bacterial cell is as low as 10 cfu ml<sup>-1</sup> (14). Therefore, because there is no permitted disinfection or sterilization process of commercially available drinking waters for removal of microorganisms, the presence of such pathogens may be of great public health concern. Because Pseudomonads constitute the main part of these both naturally occurring and contaminating bacteria (23, 9) as well as their role in opportunistic infections (24, 13), the assessment of the health risk from these organisms after drinking continues to be of high interest for both of microbiologists and health workers. Therefore, as for *Ps. aeruginosa*, their occurrence in supplied water is considered as quality indicator. Drinking water has been suggested as an important source of human infections caused by the members of *Pseudomonas* (3, 10). From health risk point of view, the great distribution of *Pseudomonas* spp. and *Aeromonas* spp. as the main components of heterotrophic bacteria in supplied water and their increased resistance to clinically available antimicrobial agents has been considered as health concern particularly to immunocompromised patient and because there is the risk of transferring the resistance to other bacteria present in the human body and some pathogenic ones (23; 18; 17). By taking into account that there are many local supplied water in the basra regions, and there is no documented and reliable information on the microbiological quality of these water products and consequently their hygienic safety for consumptions, the increased consumption of such waters raises the question as to whether

they are hygienically safe. Therefore, the aim of the study is to provide an adequate information on the microbiological quality of these water products and an assessment of their health risks through investigation of the antibiotic resistance among *Pseudomonas* spp.

## **Materials and methods**

### **Collection of samples:**

A total of 40 samples of drinking water collected from water suppliers consumed in basra at 10 different regions . These samples were stored at room temperature (20-22) C° prior to examination . All analyses tests were performed in laboratory of marine science center.

### **Bacteriological examination:**

The samples were analyzed to investigate the presence of pathogenic bacteria (*Pseudomonas* spp.) For investigation of *Pseudomonas* spp. Membrane filtration technique was applied. Different volumes up to 1 L. of samples were filtrated using cellulose membrane filter of 0.45 µm pores (Millipore's ltd) and filters were transported on to selective culture media and incubated for 72 hr at 30 C°. *Pseudomonas* agar base supplemented with 200 mg/1 cetrimide and 15 mg/1 nalidixic acid was used for detecting of *Pseudomonas aeruginosa* .

### **Identification of bacteria :**

All suspected colonies were subcultured on Tryptic Soy Agar for 24 hr at 30 C°. Each isolates was tested by Gram stain , Other confirmation tests were carried out to identify the isolates of *Pseudomonas* , these tests includes, sensitivity to vibriostatic agent O/129, motility ,indole test, methyl red test, voges proskuer test, citrate utilization test, urease test, TSI test, H<sub>2</sub>S, Gas, nitrate reduction test, catalase test, oxidase test , Carbohydrate fermentation test .

### **Antibacterial susceptibility testing:**

Using agar disk diffusing method for antibiotic sensitivity testing (Bauer et al., 1966). Bacterial isolates were suspended in sterile 0.85% saline and the cell density was adjusted to match the turbidity of McFarland NO. 2 standard, diluted in 1:20, and swabbed on Muller Hinton agar (Hi media Ltd) using sterile cotton swabs . The antibiotics and the concentration used were as follows: ampicillin 10 µg , ceftazidime : 30µg , ceftriaxone: 30µg , chloramphenicol: 30µg , ciprofloxacin: 5µg , erythromycin : 15µg , gentamicin: 10µg , colistin: 10µg kanamycin: 30µg, naladixic acid :30 µg , novobiocin: 30 µg , streptomycin : 10µg , tetracycline: 30µg . plate were incubated at 30 C° for 24-48 hr. Results of susceptibility were reported.

## **Results**

A total of 56 bacterial isolates were obtained and subjected to susceptibility tests. These isolates include *Pseudomonas* spp as shown in Table 1. In Table 1, there were 56 (14%) positive samples for occurrence of *Pseudomonas* spp.

**Table (1) : Percentage of positive samples of drinking water from suppliers in Basrah city.**

Type of sample	No. of sample	Bacteria	No. sample positive	% of
Drinking water	40	<i>Pseudomonas</i> spp.	56	14

For *Pseudomonas* spp., the highest frequency of resistance was to nalidixic acid (87.98 %) followed by tetracycline (77.9%) and ampicillin (68.82 %). (Table 2).

**Table 2. Percentage of antibiotic resistance among *Pseudomonas* spp., isolated from drinking water .**

Antibiotic	Sensitive S %	Resistant R %
Ampicillin	31.18	68.82
Ceftazidime	36.44	63.56
Ceftriaxone	82.61	17.39
Chloramphenicol	63.82	36.17
Ciprofloxacin	96.84	3.16
Colistin	86.16	13.83
Erythromycin	38.08	61.91
Kanamycin	86.16	13.83
Gentamicin	92.4	7.96
Nalidixic acid	8.12	87.98
Novobiocin	33.47	66.63
Streptomycin	42.58	57.41
Tetracycline	22.81	77.09

Five antibiotics have showed noticeable activity against *Pseudomonas* spp. These antibiotics and their sensitivity percentages are ciprofloxacin (96.84%), gentamicin (92.04%), colistin and kanamycin (86.16%), ceftriaxone (82.61%). The most effective antibiotics against *Pseudomonas* spp. were ciprofloxacin, gentamicin, colistin, kanamycin and ceftriaxone .figur 1 show the colonies of *pseudomonas* spp.

#### Discussion

Because the opportunistic pathogen *Ps. aeruginosa* is currently considered a primary infectious agent implicated in food borne and waterborne diseases (30,20). The

significance of *Pseudomonas* spp. and related species in drinking waters is related to their capability of multiplying abundantly in such low nutrient water (8; 19; 28) and surviving with somewhat constant numbers for at least 6 months (12; 15; 5). It is also worthy of noting that, *Ps. aeruginosa* has been previously suggested as a surrogate indicator for the presence of other opportunistic pathogens (7). obtained results about the predominance of *Pseudomonas* species are not fully in agreement with those obtained by many authors. In 1988, Rosenberg and Hemandez- Duquino showed that various *Pseudomonas* spp. presented in 45% of the samples from 87 different carbonated and noncarbonated drinking waters purchased in Germany (23). This proportion was decreased to 30% in a study performed by (19). Likewise, Guillot and Leclerc reported that 40% of the isolates originated from drinking water were identified as *Pseudomonas* species (9). *Pseudomonas* spp. are also abundantly isolated from most of the drinking water samples analyzed by (16). All *Pseudomonas* species identified in this study and our previous study (5) have been frequently isolated by others. Because it is not possible to recognize the potential pathogenicity of its components, HPC test is still somewhat ambiguous term and consequently it is not recommended to rely on to assess the health significance and risks associated with drinking water (6,1). Furthermore, there is no clear-cut evidence to link the gastroenteritis with heterotrophic bacteria ingested by healthy people (24 , 4). Under these circumstances, the antimicrobial sensitivity profile of the heterotrophic bacteria constituted the HPC and particularly the members of *Pseudomonas* and *Aeromonas* seems to be, at least currently, reasonable measurement in this situation. Collectively, the incidence of antibiotic resistance among aquatic bacteria is a growing area of global public health concern (22; 26; 11; 25; 29).

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## دراسة حول بكتيريا *Ps. Aeruginosa* المعزولة من مياه الشرب في مدينة البصرة.

غازي صالح المالكي ورجاء عبد الكاظم حنف

قسم الاحياء البحرية- مركز علوم البحار-جامعة البصرة

### الخلاصة

جمعت أربعون عينة من مياه الشرب لعشرة مناطق في مدينة البصرة وخلال فترة ستة أشهر من عام 2012 وحللت جرثومياً، تم عزل جرثومة *Pseudomonas* في اطباق بتري باستخدام الوسط المغذي R2A وحضنت عند درجة 22م° ولمدة خمسة الى سبعة ايام . تم التحري عن الجرثومة ومطابقتها ببيوكيميائياً. أظهرت النتائج ان 14% من عينات المياه تحتوي على جرثومة *Pseudomonas* بواقع 56 عزلة وكان القسم الاعظم من العزلات مقاومة ل ناليديكسيك أسيد، نتراسايكلين. كانت المضادات الاكثر فعالية هي سايبروفلوكساسين، جنتاميسين ، كولستين . ان المحتوى الموجود من جرثومة *Pseudomonas* في مياه الشرب تمثل تحدياً صحياً ونوعياً للمستهلك البشري . اضافة الى ان المقاومة المتعددة للمضادات يمكن ان تكون ذات اهمية صحية على الاقل عند بعض الاشخاص الحساسين ويجب ان تعمل بمزيد من الاهتمام.