USING OF PLASMID SCANNING IN EPIDEMIOLOGICAL INVESTIGATION FOR *SALMONELLA TYPHI* BACTERIA IN POLLUTED WATER SUPPLY AND PATIENTS IN AL-SADER CITY

Amina N. Al-Thwani[1] Ihsan M. Al-Saqar[2] Ashwaq B. Jasem[1]
Suham I. Ali [3] Raad S. Haneef[3] Ibtsam B. Al-Kanani [3]

[1]Genetic Engineering and Biotechnology Institute for Postgraduate Studies, University of Baghdad
[2]Biotropical Research Unit, College of Sciences, Baghdad University
[3]Ministry of Environment

ABSTRACT

A total of 452 water samples collected from Al-Sader city were examined from the 1st of January 2007 to the 1st of September 2007. The results revealed the contaminated of 169 (37.4%) samples with different types of pathogenic bacteria, 31 isolates were identified as *Proteus mirabilis*, 10 *Citobacter* spp., 15 *Streptococcus* spp., 94 *Escherichia coli*, 15 non-*vibrio cholerae* 01 and 4 as *Salmonella typhi*. While 76 isolates of *S. typhi* were isolated from 2517 patients, depending on biochemical identification and Api system epidemiological markers of the samples were studied including biotyping, serotyping and plasmid profile for both source of isolates as epidemiological index. The results of plasmid profile confirmed the similarity in the plasmid bands arrangement in all *S. typhi* isolates from patients and water samples which indicate that infection was transmitted through water.

Key words: Water pollution, Epidemiology, *Salmonella typhi.*

استخدام المسح البلازميدي في التحري الوبائي عن جرثومة *Salmonella typhi* في عينات من مياه الشرب والمرضى في مدينة الصدر

آمنة نعمة الثويني[1] احسان مهدي الصقر[2] أشواق باسم جاسم[1]
سهام ابراهيم علي[3] رعد سرحان حنيف[3] ابتسام بداي الكناني[3]

[1]معهد الهندسة الوراثية والتقنيات الاحيائية للدراسات العليا، جامعة بغداد
[2]مركز أمراض المناطق الحاره
[3]وزارة البيئـــــــة

الخلاصة

تم فحص 452 عينة من المياه المجهزة لمدينة الصدر للمدة من 2007/1/1 ولغاية 2007/9/1، أظهرت النتائج وجود 169 (37.4%) عينة ملوثة بمختلف الأنواع البكتيرية. شخصت 31 عزلة لبكتريا *Proteus mirabilis* و 10 spp. *Citrobacter* و 15 spp. *Strept.* و94 *E. coli* و 15 تعود إلى non-01 *Vibrio cholerae* و4 عزلات لبكتريا *Salmonella typhi* في حين شخصت 124 عزلة لبكتريا *S. typhi* من مجموع الـــــــــمرضى المراجعين والبالغ 2032. بالإعتماد على الاختبارات الكيموحيوية ونظام التشخيص Api Analytic profile index. تمت دراسة العلامات الوبائية Epidemiological Markers والتي تضمنت الحساسية للمضادات الحياتية والتنميط الحيوي والتنميط المصلي فضلاً عن دراسة المحتوى الوراثي والذي شمل عينات مياه الشرب والمرضى المراجعين للتحري عن مصدر الإصابة Index case في حالة الرصد الوبائي. أكدت نتائج مسح البلازميدات وجود تماثل في توزيع البلازميدات، في جميع عزلات بكتريا *S. typhi* المعزولة من المرضى والماء مما يدل على وجود عدوى منقولة عن طريق المياه.

INTRODUCTION

Most scientists have been busy in protecting their water from chemical pollution, but microbes such as bacteria and viruses pose a much greater threat. In polluted water bacterial contamination can not be detected by sight, test and smell, the only way is to have is tested. More than one billion people word wide drink unsafe water a total of 3.4 million people, mostly children die every year from water related diseases for drinking, swimming or washing clothes, vegetables… etc. in polluted water(1).

Numerous human pathogen are spread by fecal contamination of water examples, *Salmonella typhi*, *Escherichia coli*, *Giardia lambilia*, *Cryptosporedia parvum*, *Cholera* and hepatitis A. These pathogen can be a risk to human health even in low concentration. *S. typhi* remains an important cause of illness in the developing world a report by WHO indicate high infection in 1990-1998 reaches a level more than 360000 cases every year mostly in preschool children and infants(2).

Salmonellosis is regarded as one of the important communicable disease which spread in different parts of the world, its an essential problem in developing countries, where 12-15 million cases recorded annually, 7.7 million of them in Asia (3). In Iraq, *S. typhi* is responsible for a significant part of salmonella infections(4,5). It's note worthily in the etiology of out breaks and sporodic cases of typhoid fever(6).

WHO (2003) have reported 1812 cases in 1989 while the number increased to 21356 in 2004. The number of case after the last ware (2003) is expected to be much higher with collapse of health care system(6).

Case fatality is high (10-20%) in the absence of proper treatment (1).

MATERIALS AND METHODS

Samples

1- Drinking water samples: 452 water samples were collected from different locations in Al-Sader city during the period 1st of January to the 1st of September 2007.
2- Clinical samples: 2517 with sign and symptems of typhoid fever were only 2441 were positive for serological test while only 76 patients were *S. typhi*, positive depending on blood and stool culture.

Water sample analysis

A- Isolation of indicator bacteria:

Different technique were used for the analysis of water samples according to the standard methods of WHO (8) including:

1- Multiple-tube fermentation technique.
2- Fecal coli form procedure.
3- *Escherichia coli* procedure.
2- Isolation of pathogenic bacteria from water sample.

B- Isolation and identification of *S . typhi* from water samples:

Different technique used for isolation of *S. typhi* bacteria (8,9,10)

C- Isolation and identification of fecal *Streptococcus*

According to WHO (8) to differentiate human fecal contamination from that of other warm- blooded animal.

3- Isolation of *S. typhi* from clinical samples:

From 2517 patients with sign and symptoms of typhoid fever, *S. typhi* bacteria were isolated from blood culture, stool culture in addition to biochemical and serological tests (11).

4- Antibiotic susceptibility:

Antibiotic Sensitivity of clinical and environmental isolates were screened by agar diffusion method (12) using the following antibiotic discs: Tetracycline, Amoxicillin, Cotrimoxazole, Chloramphenicol-1, Nalidxicacid, Ciprofloxacin, Ceftriaxone.

5- DNA extraction of bacteria isolates from clinical and water samples:

Genomic DNA was extracted from bacterial culture of *S. typhi* using two different methods the alkalyine lysis (13). All *S. typhi* isolates were sereend for plasmid content. The second method using wizard Genomic DNA purification kit promega (USA).

6- Detection of DNA content: Horizontal gel electrophoresis was used for detection of DNA while visualized with the aid of ethedium bromide and U.V. transilluminater (14).

RESULTS AND DISCUSSION

1- Water samples

Four hundred fifty two drinking water samples were analyzed and examined for *S. typhi* and other pathogenic bacteria, 169 polluted sample with 31 isolates of *Proteus mirabilis*, 10 *Citrobacter*, 15 fecal *Streptococcus*, 94 *Escherichia coli*, 15 non-*Vibirio cholerae* and 4 isolates of *Salmonella typhi* (Table 1).

Table (1): Different bacteria isolates from water samples.

Month	No. of sample	No. of bacteria isolates						
Jan.	35	10	0.23	5.5	0.59	1.8	0.88	0.88
Feb.	28	5	0.11	2.7	0.29	0.9	0.44	0.44
March	50	18	0.4	10	1	3.3	1.59	1.59
April	70	30	0.7	16.6	1.77	5.5	2.6	2.6
May	30	7	0.16	3.8	0.35	1.2	0.6	0.6
June	34	8	0.18	4.4	0.47	1.4	0.7	0.7
July	68	27	0.63	15	1.59	4.9	2.3	2.3
Aug.	67	31	0.73	17.2	1.83	5.6	2.7	2.7
Sept.	70	33	0.78	18.3	1.95	6	2.9	2.9
Total	452	169	1.4	35.1	3.7	11.5	5.6	5.6

This study showed an increment of water contamination especially in the end of summer, which may be due to suitable temperature that enhance the propagation and growth of bacteria. Al-Hindawi(16) recorded that *Salmonella* spp. appeared in spring more than any other seasons, while, Al-Hayali (5) in other survey, indicated that the highest presence of *Salomonella* spp.was in summer and autumn. He mentioned that bacteria had the ability to survive weeks in cold water and in the sewage, also boiling water, and can survive and multiply in milk and it's products in favorable condition, while Hassan (16) was unable to isolate *Salmonella* spp. from 125 water samples, which were culture in the period from beginning of June 2003 till the end of December 2003.

2- Clinical samples

Based on biochemical findings, 76 isolates of *S. typhi* were characterized from fecal and blood samples collected from 2517 patients who attended the hospitals with signs and symptomes of typhoid fever and diarrhoea with abdominal pain (Tables 2,3) samples in Al-Sader city hospitals.

Table (2): Incidence of bacteria which recovered from blood.

Month	No. of patient	Widal test	blood culture
Jan.	91	89	1
Feb.	123	120	2
March	101	96	4
April	421	408	7
May	562	547	12
June	620	609	7
July	151	143	7
Aug.	321	310	9
Sept.	127	119	7
Total	2517	2441	56

Table (3): Incidence of bacteria which recovered from stool samples in Al-Sader city hospital

Month	Fecal sample	*Shigella flexnerie*	*E. coli*	*S. typhi*	*V. cholerae*	Percentage
Jan.	70	3% 4.2	1% 1.4	1% 1.4	-	(5) 0.9%
Feb.	72	5% 6.9	2% 2.7	1% 1.4	-	(8)1.45%
March	40	3% 7.5	7% 17.5	1% 2.5	-	(11)2%
April	42	1% 2.3	2% 4.7	2% 4.7	-	(5)0.9%
May	60	2% 3.3	3 5%	5% 8.3	-	(5)0.9%
June	50	2% 4	3% 10	1 2%	-	(10)1.9%
July	52	2% 3.8	4% 7.6	2 3.8%	-	(8)1.4%
Aug.	84	6% 7.1	4% 4.7	2 2.3%	-	(12)2.1%
Sept.	86	7% 8.7	4% 5	5% 6.2	-	(16)2.9%
Total	550	31% 37.3	32% 38.3	20% 24	-	51%

Eighty three (15%) of samples were positive for different types of bacteria, higher infection was recorded in September (16) 2.9%, the lowest observed in January (5, 0.9%).

S. typhi detected in high percentage (4%) especially in May (8.3%) while in January it reaches (1.4%) *E. coli* and *Sh. felxneri* had a proximally the same high percentage (32.5% and 31.3%), respectively. Also the highest infection was recorded in March for the first (17.5) and for the second in September (8.7) while the lowest was recorded in April for *Sh. flexneri* (2.3) and for *E. coli* in January (1.4).

In this study, biotyping depending on the biochemical reactions in Api 20 E system showed two biotypes which differentiated by their ability to decarboxylate lysine. Fifty six isolates from clinical samples were lysine (+ve) except (20) were lysine (-ve), while only one isolate from water samples was lysine (+ve) and (3) isolates were lysine (-ve).

This variation may be due to gene expression which made the same strains appear one or more biochemical reactions, random mutations may also confound the interpretation of these data.
These results agreed with Al-Hindawi (17) as shown in Fig. (1).

Fig. (1): Represent photograph of biochemical reactions of *S. typhi* with Api 20E strep.

3- Sensitivity Testing

Antibiotic sensitivity test of the 76 clinical isolates and 4 environmental isolates of *S. typhi* were screened by agar diffusion method (Table 4). The results indicated that all clinical and environmental *S. typhi* showed either sensitivity or resistance to antibiotics used.

Table (4): Susceptibility of *S. typhi* to different antibiotic.

Antibiotics	Enviromental isolates			Clinical isolates		
Cortimoxazole	S	I	R	S	I	R
	2	-	2	50	4	26
Amoxcilline	1	-	3	9	6	61
Ciprofloxacin	1	1	2	76	-	-
Chlorampheni col-1	1	1	2	55	-	21
Tetracyctine	1	-	3	6	3	67
Nalidixic acid	-	-	4	31	-	45
Ceftriaxone	-	-	4	72	-	4

S= Sensitive R= Resistant I= Inter mediate

Three isolate of *S. typhi* which had recovered from water samples showed high resistant (1.7%) to tetracycline while 1 (0.57%) isolate appeared to be sensitive to this antibiotic. The isolates gave high resistant 3 (1.7%) to Amoxicillin also, while resistance reached 2 (1.14%) and 2 (1.14%) against cortimoxazole and chloramphinical respectively.

Nearly similar result of percentage of resistance and sensitivity were noticed when *Salmoneela* treated by cotrimoxazole and were 2 (1.14%) and 2 (1.14%), respectively. The bacteria appeared to be more resistant 4 (2.28%) to nalidxic acid.

All isolates appear sensitive (100%) to ciprofloxacin while in *S. typhi* recovered from patients, 76 isolates appear sensitive to ciprofloxacin and cortimoxazole 50, chloramphenicol-1 55 also ceftriaxone 72.

Depending on the antibiotic sensitivity, our findings revealed that most of the isolates were marked by multidrug resistant (MDR), and that may be attributed to the continuous use of antibiotic for treatment of typhoid fever this phenomena pause a big challenge to the scientists when the resistance to antibiotic increased and make a global problem. Reports of WHO (8) confirmed the spread of disease by *Salmonella* resistance bacteria. The main cause of the appearance of MDR is due to the presence of the plasmid in bacteria which carries the gene of resistance to antibiotics which increases with time. Some scientists confirmed that the random use of antibiotics against Salmonella in the community create resistance problem and increase the death due to in sufficing treatment. Salmonella is a big health problem in developing countries in Asia, Africa and Latin America due to suitable environmental condition for the survival and growth of the *Salmonella*, beside unclean food and water supply (18).

4- Plasmid profile

The detection of plasmid profile considered one of the characterization techniques which is based on the genetic content of strains and shows the relationship between them (19). DNA was extracted from clinical and environmental isolates by standard alkaline lysis procedure (13). The results of the method reveale that majority of isolates (52) were free of plasmid while only (24) isolates carried plasmid which is similar in arrangement in all *S. typhi* isolates from patient and water samples which indicate that infection was transmitted through water (Fig 2).

This observation is in a good agreement with the results observed by others (6,21).

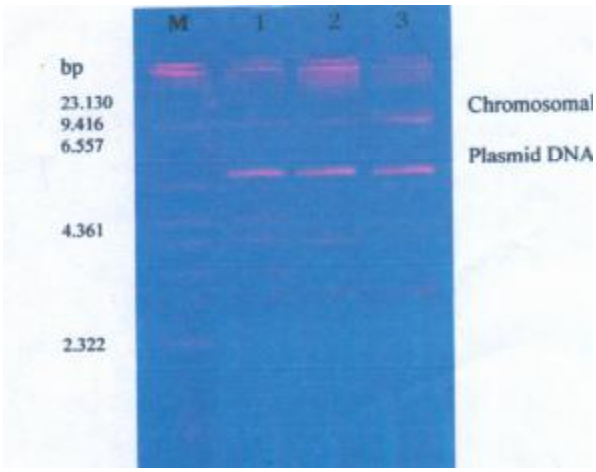

Fig. (2): Agarose gel electrophoresis of plasmids extracted. Lane M: λ DNA cut with HindIII; 1-3 patterns of Salmonella typhi plasmid DNA extracted from patient and water samples (1% agarose, 60v., 1-2hrs).

REFERENCES

1. World Health Organization (2003). Communicable disease profile for Iraq. Viena.
2. Crump, J. A.; Luby, S. and Mintz, E.(2004). The global burden of typhoid disease. bull. *World Health Organization*, 82: 346-353.
3. Todour, K. (2005). Salmonella and salmonellosis. Tudors online Textbook of Bacteriology Madison Department of Bacteriology, University of Wisonson.
4. Al-Ani, Z. I.(1976). *Salmonella* curriers in Baghdad. M.Sc. thesis. Medical Microbiology, College of Medicine, Baghdad University.
5. Al-Hayali, G.(1993). Salmonellosis in Iraq, five- year review 1985-1989. Diploma, Medical Microbiology. College of Medicine, Al-Nahrain University.
6. Faik, A. J.(2007). Molecular epidemiology analysis of *Salmonella entterica* serotype Typhi. Ph. D. thesis, College of Medicine, Al-Mustanseria University.
7. World Health Organization (1998). Standard Methods for Examination of water and waste water 20ed.
8. Baumann, P. and Schubert, R. H. W.(1984). Family II vibrionaceae, In: Bergeys manual of systematic bacteriology (ed. N. R. Kreig and J. G. Holt). Vol, 1. Md. pp. 518-538. The Williams and Wilkins Co., Baltimore.
9. Elliot, E. L.; Kaysner, C. A. and Tamolin, N. L.(2001). *Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and other *Vibrio* spp. Bacteriological analytical manual online. Chapter 9. Center for food Safety and Applied Nutrition.
10. World Health Organization (2002). *Vibrio cholerae*. Addendum: Microbiological agents in drinking water quality.
11. Jawitez, E.; Melnick, J. K. and Adelberg, E. A.(1998). Review of medical microbiology. 21st ed. Middle East Edition, Beirut, Lebanon.
12. Sambrook, J.; Fritsch, E. F. and Maniatis, T.(1989). Molecular cloning: a laboratory manual, 2nd ed., cold spring Harbor laboratory press, cold spring Harbor, N. Y.
13. Birnboim, H. C. and Doly, J.(1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.*, 7: 1513-1523.
14. Lawyer, F. C.; Stofell, S.; Saiki, R. K.(1993). High level expression, purification and enzymatic characterization of flue length Thermus aquatics DNA polymerase and truncated form deficient in 5-3 exonuclease activity. *PCR Methods Appl.*, 2: 275-287.
15. Bauer, A. W.; Kirby, W. M.; Sherris, J. C. and Turk, A.(1966). Antibiotic susceptibility testing by standardized single disk method. *American J. Clin. Pathol.*, 45: 493-496.
16. Al-Hindawi, I.(1986). Cited from, Ramadan, A. T. (2006). Epidemiological and genetic study of the causes of typhoid fever thesis, College of Science, Baghdad University.
17. Hassan, A.(2004). Epidemiological and molecular study of antibiotic –Resistance of some Salmonella species isolated from clinical and non-clinical specimens, MSc. Thesis, College of Science, Baghdad University.
18. Wikipedia (2001). Salmonella from wikipedia the free encyclopedia, USA.
19. World Health Organization (2006). 6th International Conference on Typhoid Fever and other Salmonellosis. Geneva, WHO.

20. Popovic, T.; Bop, C. A.; Olsuik, O. and Waschsmuth, K.(1993). Epidemiological application of a standardized ribotype scheme for *Vibrio cholerae* O1. *J. Clin. Microbiol.*, 31: 2474-2482.
21. Murray, B. E.; Levine, M. M.; Cordano, A. M.; D'Ottone, K.; Jayanetra, P.; Kopecko, D.; P-Urae, R. and Preuzol, I.(1985). Survey of plasmids in *Salmonella typhi* from Chile and Thailand. *J. infect Dis.*, 151(3): 551-555.