EPIDEMIOLOGICAL STUDY OF SALMONELLA TYPHI OUTBREAK IN BAGHDAD [+]

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Abstract:

Through the period extending from the 1st till the 27th of November, 2002, blood samples were collected for culture from 209 patients attending two health centers at Al-Sadder, a large province of the capital Baghdad, complaining of fever. Forty eight samples yielded positive results with the isolation of *Salmonella typhi.*

Epidemiological study was planned to demonstrate possible source of infection. Markers used were Antibiogram, Biotyping, and plasmid profile.

All isolates were susceptible to the majority of antibiotic tested. One pattern of biochemical reactions was seen. Plasmid profiles showed at least three patterns.

المستخلص:

خلال الفترة الممتدة بين الاول من تشرين الثاني ولغاية ٢٧ تشرين الثاني لعام ٢٠٠٢ سحبت عينات الدم لغرض الزرع من ٢٠٩ مريض راجعوا اثنين من المراكز الصحية في منطقة الصدر ، والتي تعتبر مقاطعة كبيرة في محافظة بغداد ، يشكون من الحمى . ٤٨ عينة أعطت نتيجة موجبة لزرع بكتريا Salmonella typhi .

صممت الدراسة الوبائية لملاحظة احتمالية مصدر التلوث . المعلمات المستخدمة كانت دراسة استجابة العزلات لمضادات الحياة ، والتنميط البايولوجي ، والعرض البلازميدي .

جميع العزلات كانت حساسة لمعظم المضادات المختبرة . ولوحظ ان جميع العزلات اعطت نمط واحد من التفاعلات البايوكيميائية . وقد اظهر العرض البلازميدي وجود ثلاث انماط على الاقل .

Introduction:

Salmonella typhi is still a major cause of morbidity and mortality in many parts of the world, particularly Asia, Africa, and Latin and South American [1]. There are estimated to be at least 16 million cases of enteric fever annually [2] and in some

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areas Salmonella typhi or Salmonella Paratyphi A or B are still the most common cause of community acquired bactermia [3,4] . In India a high mortality rate was demonstrated [5]. Large number of epidemics with this organism has been reported with different sources of infection [6, 7]. Many local studies and reports accentuates that water-borne epidemics today in very old cities in Baghdad, particularly al-Sadar and al-Aurfaly are the result of failure to make use of the available existing knowledge and technologies. Thus, the water pipes are old and the sewage pipes leak, so the sewage has easy access to the water pipes at a point just before it enters the dwelling. In addition, untreated raw sewage and industrial wastes are dumped directly into local waters; then, a storm or flood is resulting in the local drinking water being contaminated with sewage. Finally, the pathogens of cholera, typhoid fever, bacterial and amebic dysentery, giardiasis, infectious hepatitis can all be spread through contaminatewater[8,9,10].Monitoring such epidemics requires the identification of clones of organisms which can be traced geographically and temporally to investigate the transmission of Salmonella from a common source [11].Nonmolecular laboratory techniques for typing Salmonella are phage typing, biotyping, serotypings, colicin typing and antibiogram [12]. Of the molecular techniques, analysis of plasmid DNA is the basis of most epidemiological studies. Restriction endonuclease analysis has its applications when no plasmids are present or plasmids are of the same size in the strain though they may have eventually different DNA sequences. Southern blot hybridization in another technique that could be of value [13].

Material and Methods:

Through the period of 27 days in November 2002, blood samples were collected from 209 patients attending the Al-Obaidi (61 patients) and Al-Aurfali (148 patients) primary health centers complaining of fever after examination by the general practitioner in these centers. Five milliliter of patient's blood was inoculated into blood cultures bottles containing 45ml of brain heart infusion broth [14]. The blood culture bottles were incubated for 6-18 hours at 35^{o}C. Macroscopic changes were observed next day. Gram stain was performed irrespective of macroscopic evidence of growth. Blind subcultures on blood agar, MacConkey agar, and Choclate agar were carried out. Isolates were identified using API – 20 E systems (bio Merieux).

Laboratory Investigations:

Antimicrobial susceptibility testing: was done using the Bauer-Kirby method [15]. Disks used were; ampicillin, chloramphenicol, gentamicin, ciprofloxacin, cefotaxim, and co-trimaxazole. Three or four colonies were picked from overnight incubated culture plate (MacConkey agar) and inoculated into 4ml of Trypticase Soya broth (Biomerieux). The culture tubes were incubated at 37^{o}C for 3-4 hours. Their optical densities were then adjusted to match Barium Sulphate standard turbidity (0.5 ml of 1% Barium chloride to 99.5ml of 1% 0.36N Sulphuric acid) by addition of sterile saline. A sterile disposable cotton swab was soaked in the adjusted broth culture used to inculate freshly prepared Muller-Hinton agar plate (Oxoid). After the inoculums dried, the antimicrobial disks were applied. The plates were incubated within 30 minutes for 18-24 hours at 37^{o}C.

Plasmid profile: The large scale plasmid extraction method [16] was used for extraction of plasmid DNA from the isolates.

The extracted plasmids were separated by horizontal electrophoresis (5 volt /cm for 2-3 hours) using 0.7% (w/v) agarose gel and TBE buffer (0.089 M Tris , 0.089 boric acid and 0.002 M EDTA) .

Gel was stained with ethedium bromide and photographs were taken under UV trans-illumination at 340 nm [17] .Plasmid pBR 322 was included in all profiles for determining the size of plasmids (reference DNA).

Epidemiological investigations: Patients age, sex, date of admission, site and residential area were recorded. A written questionnaire including possible risk factors as drinking non-municipal water , drinking unboiled water , eating food from a street vender and consumption of a particular food (milk , cheese) .

Environmental investigation: To evaluate municipal water, samples were collected from taps at different houses of affected patients and their neighborhood as well as randomly selected samples. Twenty six samples were examined from Al-Obaidi district as well as 41 samples from Al-Aurfali district. All these samples were examined by the laboratories of Amanat Baghdad for the presence of fecal coliform bacteria.

Results:

Of the 209 patients included in this study .Ninety three were female patients and 116 were males. Their age range from less than 5 to more than 30 years with predominance of younger age group. Figure 1 shows the age distribution of patients enrolled in the present study of the total cases. Ten blood cultures were positive for Salmonella typhi at Al-Obaidi district and 38 at Al-Aurfali, giving total positive cases of 48 with Salmonella typhi bacteremia.

Biochemical reactions: Using API – 20E system (which contains 20 different biochemical reactions including 10 enzymatic reactions and 10 fermentation / oxidation reaction). One biotype was seen.

Antibiograms: show susceptibility of the tested isolates to the antibiotics used.
Plasmid profile analysis: Figure 2 shows the three patterns seen (A, B, and C). It demonstrates the profiles of 14 of the isolates tested in lanes 1-14. Lane 15 shows the standard pBR322 .The first pattern (A) contains no plasmid. The second pattern (B) contains three plasmid bands which are parallel to the third band of the reference DNA.The last pattern (C) contain two plasmid bands; one of them was mega (large) plasmid.

Environmental investigation: Water samples were taken from water treated plants yielded negative results. However, water samples collected from taps yielded the presences of fecal coliform bacteria in 18 to 69 houses hold tap water. Since, of the 26 water samples collected from Al-Obaidi district, five showed unaccepted high colony count of fecal coliform bacilli. Of the 41 water samples collected from Al-Aurfali district, 13 were significantly contaminated with coliforms .World Health Organization guidelines for potable water require les than 1 colony forming unit (CFU)per 100 ml (WHO)[18].

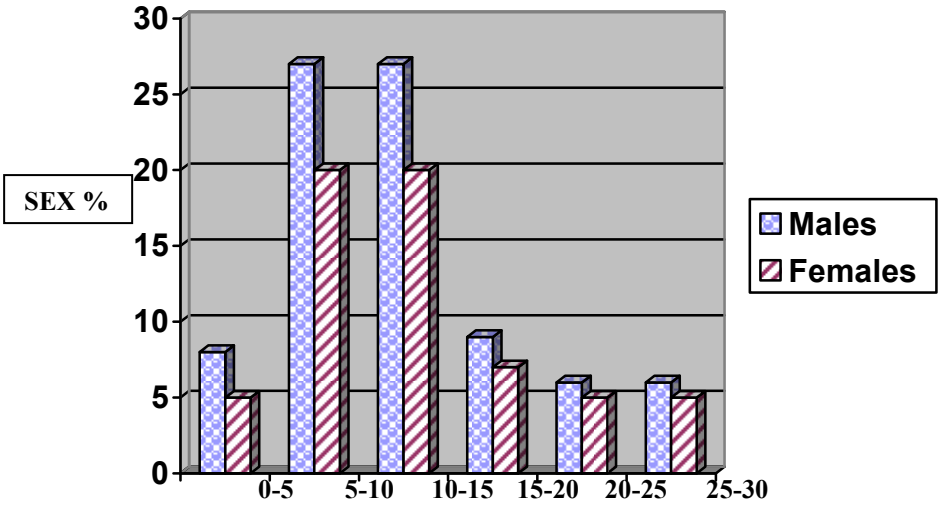

Figure 1: Age and Sex distribution of patients enrolled in the study.

15	14	13	12	1 1	10	9	8	7	6	5	4	3	2	1	Lain
	A	C	A	A	C	C	B	C	A	A	A	B	A	C	Pattern

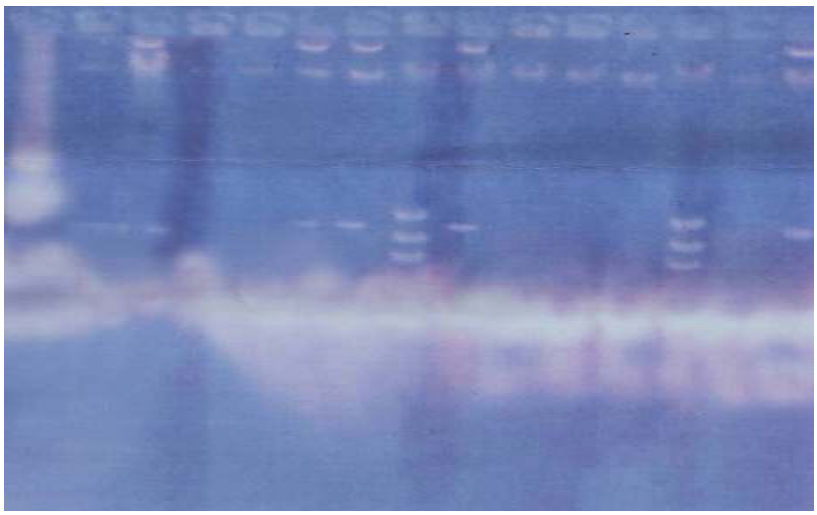

Figure 2: Plasmid profile pattern of some of the isolated bacteria

Discussion:

Salmonella Typhi in a sector of Al-Sadr city made the central public Health laboratory in accordance with the Department of preventive medicine in the Ministry of Health to take measures to asses the
Situation. Thus, staff of the laboratory was asked to take blood samples for culture from suspected cases with pyrexia from Al-Obaidi Health center. The work extends to involve Al-Aurfali Health center with the emergence of a similar situation.

According to the results of the examination of water samples, the distribution of cases over a large area within a short time favors the possibility of water-borne disease.

Phage typing is still the most common laboratory test used in investigation of Salmonella epidemics. Due to the lack of this test in our laboratory we tried other tests that could be used in such a situation. Of the conventional techniques used were serotyping in which all isolates showed positive identification of Salmonella typhi.

Biotyping, relying on the 20 biochemical reactions in API-20E showed one pattern of biochemical reactions.

Antibiogram , surprisingly showed susceptibility to a wide range of antibiotics used , a point of particular interest since we are aware of increasing incidence of multiple drug resistant (MDR) Salmonella typhi [20,21] . These results continue to be bewildering since the plasmid profile showed at least three different patterns. However, other characters may encoded by these genes, a possibility that could be checked by genetic exchange with new organisms.

Bacteria often contain extrachromosomal genetic elements called plasmids; some plasmids encode products that provide a survival edge such as determinants of antimicrobial resistance [22]. Most drug resistance in enteric bacteria (Salmonilla) is attributable to the widespread transmission of resistance plasmids [23] .Based on this principal, from plasmid profile (Figure 2), pattern A isolates must has sensitivity for most antibiotics used in the present study. In another hand , from the result of pattern C and D, it has suggested that these isolates has plasmids carry genes for resistance of one and several antimicrobial drug (for determination of which plasmid of these carry the antibiotic resistance gene needs a wider study).

This result indicates that the possibility of an outbreak with a single clone is unlikely, thus the most probable hypothesis is that the municipal drinking water is the source of bacteria in this epidemic and that contamination of water occurs at multiple sites. This hypothesis is strengthened by the finding of unacceptable number of coliform bacteria in the tap water at houses. The presence of more than one bacterial profile suggests multiple contamination sites. Possible reasons for contamination of water:

1- Damage in the distribution system allowing wastage water to enter the supposedly closed distribution system.
2- Low water pressure in the distribution system, by the supplier or due to the use of pumps for water aspiration from the distribution system, contributed to the cross-contamination of drinking water with waster water. Possible damage in the water treatment plants due to the shortage of essential requirements for proper treatment of water. This could include inefficient overused fillers which might be ineffective at removing solids and biological contaminants from river water. Other possibility might be inadequate chlorination of water.

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