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An Outbreak of Lactose Fermenter Multidrug Resistant *Salmonella enterica serova typhi* in Sulaymani City, Iraq

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Abstract: In order to present the clinical microbiology characteristics of outbreaks of lactose fermenting *S. enterica serova typhi*, thirty lactose fermenting (L+) and three nun-lactose fermenting (L-) *Salmonella entereca serova typhi* were isolated from blood of patients suffering from typhoied fever in indor and outdor of Sulaymani city-Iraq. The isolated *S. enterica* exihibited same charecterstics as the type strain and L-*S. enterica* except for ONPG production. Serological examination reveled that all L+, L- and standered strain possesed the antigenic formula as *S. enterica*. The apperence of all L+*S. enterica* colonies on different media was similar to that of *E. coli.* The L+*S. enterica* isolates were 100% resist to Ampicillin, Cefalosporin, Chloramphenicol, Gentamycin, Tetracyclin and Trimethoprim antibiotics, While L-were sensitive to Amikacin, Cefalosporin, Cefotaxim, Ciprofloxacin, Chloramphenicol and Gentamycin. However 43.3% of L+*S. enterica* isolates were resist to all tested antibiotics, 6.7% were resist to 10 antibiotics out of eleven, 30% resist to nine antibiotics, 6.7% resist to 8 antibiotics, while 13.3 were resist to seven antibiotics.

Key words: Antibiotic resistant, *E. coli*, lactose fermenter, *Salmonella typhi*, sulaymani, typhoid fever

INTRODUCTION

Typhoid fever caused by *Salmonella enterica serovar typhi* remains endemic to many parts of Iraq, including Sulaymani city. To differentiate *Salmonella* from other *Enterobacteriaceae*, bacteriologists use lactose fermentation as a key biochemical test. As early as 1887, it was known that *Escherichia coli* was alactose fermenter (L+) and that *Salmonella* was not a lactose fermenter (L-). Therefore, most differential plating media commonly developed and used today for the isolation of *Salmonella* contain lactose (Ewing *et al*., 1986; Janda and Abbot, 1998). It has been reported that less than 1% of all *Salmonella* ferment lactose (Patrick *et al*., 2000). There have been various reports of the occurrence of (*Lac+*) multidrug resist *Salmonella* in human (Chassy *et al*., 1978; Corbion, 1981; Ezaki *et al*., 1987; Camara *et al*., 1989; Coovadia *et al*., 1992; Patrick *et al*., 2000; *Eswarappa et al*., 2009). Until recently, the majority of *S. enterica isolates* from Sulaymani remained susceptible to Ceprofloxacin, Gentamycin and Amikacin, and only some cases of typhoid due to *Lac+* multi-antibiotic resistant isolates of *S. enterica* have been documented, and therefore, these antibiotics still recommended as first line therapy for patients with typhoid fever.

Since 1907, there have been various reports of the occurrence of lactose fermenting *Salmonella* in humans, such as *Lac1Salmonella enterica* serotype Virchow, *S. enterica* serotype Tennessee, *S. enterica* serotype Indiana, *S. enterica* serotype Agona, *S. enterica* serotype Typhimurium, *S. enterica* serotype Oranienburg, *S. enterica* serotype Tuebingen, *S. enterica* serotype Newport, *S. enterica* serotype Typhi, *S. enterica* serotype Java, and *S. enterica* serotype Toulon (Camara *et al*., 1989; Kukulska and Zimmer, 1989; Coovadia *et al*., 1992; Louie *et al*., 1993; Usera *et al*., 1996; Usera *et al*., 1998; Patrick *et al*., 2000).

It is apparent that there are no reports that convey details of outbreaks caused by Lac+ multi-resistant strains of *S. enterica serova typhi* in Sulaymani city. The purpose of our work is to present the clinical microbiology characteristics of outbreaks of lactose fermenting *S. enterica serova typhi* in human blood suffering from typhoid fever in the middle of 2009 in indoor and outdoor patients in Sulaymani city, Iraq.

MATERIALS AND METHODS

Bacterial isolates: The isolates of *S. enterica* used in this study originated from blood of indoor and outdoor patients suffering from typhoid fever from General and Teaching Hospitals in the middle of 2009 in Sulaymani city Iraq.

Standard strain: The standard strain *Salmonella enterica* NCTC12023/ATCC14028 Kindly provided by Media Diagnostic Center, Erbil, Iraq was used as reference strain in the study.

Table 1: Some biochemical tests of *S. entereca*

Bacteria	Sources of isolates	Oxidase	Kliglar (slope)	Kligler (bottom)	H_2S production	Minitol fermentation	Acid formation Xylose	Indolformati	Lactose fermentation
30 L+ *S. typhi*	Blood	–	Red alkaline	Acid yallow	Little H_2S	+	+	+	+
3 L- *S. typhi*	Blood	–	Red alkaline	Acid yallow		+	+	+	–
Standard strain	MCI	–	Red alkaline	Acid yallow		+	+	+	–

+: Positive; -: Negative

Bacteriology: Acid production from carbohydrates was tested in purple broth base, supplemented with 0.1 volume of a sterile 10% solution of each carbohydrate. The colonies were then screened for H_2Sproduction and concurrently for lysine decarboxylase activity. Screening for H_2S production (black precipitate in the medium) was done with sulfide-indole-motility agar medium, and Kligler agar (Andrews and Hammack, 2000). In addition a commercial identification system API 20E system was used.

Serological examination test: Direct testing of colonies by slide agglutination tests were performed by using O-antisera, H and Vi antisera according to (Andrews and Hammack, 2000).

Standard bacterial strain *Salmonella enterica* NCTC12023/ATCC14028 was used as quality controls in these assays.

Susceptibility to antimicrobial agents: The susceptibility of the thirty lactose fermenting *S. enterica* isolates and three lactose-non fermenting colonial dissociate against eleven antimicrobial agents (Amikacin Ak, Ampicillin Amp, Cefalosporin Cef, Cefotaxim Cfm, Ciprofloxacin Cip, Chloramphenicol Chl, Gentamycin Gm, Rifampicin Rif, Striptomycin Str, Tetracyclin Tet and Trimethoprim Tri) was determined by means of disk diffusion method (Atlas *et al*., 1995).

RESULTS

The thirty lactose-fermenting isolates (L+) and three nun-lactose fermenting isolates (L-) exhibited the same characteristics as the type strain of *S. enterica* except for ONPG production, These L+ isolates were produced acid from lactose in purple broth base and were positive in O-nitrophenyl-beta-D- galactosidase (ONPG) test in the API 20E system. The L-isolates show an acid butt and alkaline slant on kligler (Kl) agar with small quantity of H_2S visible in the Butt, these isolates produced no acid in purple broth base supplemented with lactose and gave a negative reaction in the ONPG test. Moreover the profile number obtained for API 20E test from the L+ isolates (7404543) were identified correctly. Standard strain was negative lactose fermented with the profile number of API 20 E test (6404540).

Acid from xylose for all L+, and L- isolate was positive Table 1. The lactose fermenting isolates consisted of motile, oxidase-negative, gram-negative rods. They produce colonies on MacConkey agar and turned triple sugar iron agar yellow (Butt and slant) without gas formation. Indol production was observed on suitable media, these L+ isolates produced a small quantity of H_2S when grow on KI agar Table 1.

Serological examination revealed that all lactose fermenters, three nun fermenting-lactose and standard strain possessed the antigenic formula of *S. enterica.* Thus, all lactose-fermenting isolates were identified as strains of *S. enterica serova typhi,* and the febrile illness was diagnosed as typhoid fever caused by lactose-fermenting *S. enterica serova typhi.*

The overall appearance of the *Lac+ S. typhi* colonies on different media was similar to that of *E. coli*, i.e., they appeared as rough, flat, lactose-fermenting bacterial colonies. All isolates recovered from plating on MacConky agar yielded rough, red colonies, and on Levine eosin-methylene blue agar, the colonies looked like small, rough, 2-4 mm green sequins with metallic sheen.

The L+ isolates were 100% resist to Amp, Cef, Chl, Gm, Tet and Tri antibiotics, whereas the L- dissociate was sensitive to, Ak, Cef, Cfm, Cip, Chl and Gm antibiotics Table 2.

DISCUSSION

Thirty isolates of lactose fermenting *S. enterica serova typhi* were isolated from blood of in patients and outpatients of teaching and general Hospitals, in Sulaymani city, Iraq. Lactose-fermenting *S. enterica* isolates obtained as the etiological agent of patients suffering from typhoid fever. All these isolates when identified were exhibited the same characteristics as the non-lactose fermented *S. enterica,* and standard strain except for β-galactosidase (ONPG) enzyme production, these colonies were similar to that of *E. coli*. The β-galactosidase enzyme produced by strains of *Lac+Salmonella* differs from *E. coli* β-galactosidase, offering further evidence that the operon did not originate from *E. coli* but could have originated from an enterobacterial ancestor common to *E. coli* (Cornelis, 1981; MacDonald and Riley, 1983; McClelland *et al*., 2001). Bacteria belonging to the genus *Salmonella* are closely related to those belonging to the genus *Escherichia* and they have diverged from a common ancestor about 100 million years ago (Doolittle *et al*., 1996). Despite their close relationship, *E. coli* has more than 800 genes that are absent in the *S. enterica* genome and more than 1, 100 *S. enterica* genes lack their homologues in *E. coli* (McClelland *et al*., 2001). The

Table 2: Antibiogram of *S. entereca* isolated from blood of patient seafaring from typhoid fiver

Antibiogram groups of L+ *S. typhi*	Isolates number	Antibiotics										
		Ak	Chl	Amp	Cef	Cfm	Rif	Gm	Cip	str	Tet	Tri
1	5,8,9,11,12,15,18,19,21,24,26,27,28	R	R	R	R	R	R	R	R	R	R	R
2	3,10	S	R	R	R	R	R	R	R	R	R	R
3	20,25	R	R	R	R	S	S	R	R	R	R	R
4	7,13,16,17,22,29,30	S	R	R	R	S	R	R	R	R	R	R
5	14,23	S	R	R	R	S	S	R	R	R	R	R
6	2	S	R	R	R	R	S	R	S	S	R	R
7	1,4,6	S	R	R	R	S	S	R	S	R	R	R
L- *S. typhi* isolates		S	S	R	S	S	R	S	S	R	R	R

R: Resist to antibiotics; S: Sensitive to antibiotics

region containing *Lac*+ and *Lac* operon is one such locus and is present in *E. coli*, but absent in *S. enterica*. Thus *E. coli* is a lactose fermenter, whereas *S. enterica* is lactose non-fermenter. Nonetheless, diseases caused by lactose fermentation in extra-chromosomal genetic elements like plasmids (Patrick *et al*., 2000; Eswarappa *et al*., 2009), these elements can be horizontally transferred and acquired by bacteria (Hensel, 2004). The *Lac* operon consists of three genes, *lacZ*, *lac Y* and *lac A* which encode β-galactosidase (Wilson *et al*., 2007). In this study and during the diagnosing it have been through that these isolates are resembling the *E. coli*.

Kohabata *et al*. (1983) thought that these L+ *S. enterica* isolates were to be identical to a strain 643 *Lac*+ harboring the (*Lac*+) plasmid from ST-2 strain, strain 643 *Lac*+ was derived from typical *S. enterica* 643 treated with the episome of strain ST-2 during the course of a genetic experiment. Thus, there have been some Salmonellosis reports of typhoid fever due to a naturally occurring *Lac*+ *S. enterica* from different countries (LeMlnor *et al*., 1974; Ananel *et al*., 1980; Yanazaki and Kubota, 1982; Coovadia *et al*., 1992; Patrick *et al*., 2000; Wilson *et al*., 2007).

The environment contain not only salmonellae but also other enteric bacteria, such as *Klebsiella pneumoniae* (MacDonald and Riley, 1983; Lucain and Piffaretti, 1983; Walia *et al*., 1987), *Lactobacillus* (Chassy *et al*., 1978), *Proteus* (Walia *et al*., 1987), and *Serratia* (Walia *et al*., 1987), which may carry the lactose operon on a plasmid or, in the case of some *Klebsiella* strains, on the chromosome (MacDonald and Riley, 1983). One theory for the origin of *Lac* in these plasmids involves the unlikely transfer of the operon from the chromosome of another enteric bacterium (*E. coli*) via a transposon (Cornelis, 1981) Some strains of *Klebsiella* carry the *lac* gene on the chromosome, there is evidence for may contain determinants of antimicrobial resistance, and there have been reports of outbreaks of *Lac*+ and antimicrobial agent-resistant *Salmonella* in the literature (Timoney *et al*., 1980; Threlfall *et al*., 1983; Ezaki *et al*., 1987). However 43.3% of our lactose fermenting *S. enterica* isolates were resist in vitro to all antimicrobials used, 6.7% were resist to 10 out of eleven antibiotics, 30% resist to nine antibiotics, 6.7% resist to 8 antibiotics, while 13.3 were resist to seven antibiotics Table 3. All L+*S. enterica* isolates were 100% resist to Chl, Cef, Amp, Gm, Tet, and Tri, whereas the L-*S. enterica* isolates were sensitive to Ak, Cef, Chl, Cfm, Cip and Gm, and in some cases resistant to Chl. Multi-drug resist *S. enterica* which is reported in this study seems to confirm other reports of other workers (Nadgir *et al*., 1998; Shrikala *et al*., 1999; Sanghavi *et al*., 1999; shrigala, 2004).

Table 3: Resistant of L+ *S. entereca* isolates to use antibiotics

% of L^+ *S. typi* isolates	Antibiotics
43.311	11
6.710	10
30.09	9
6.78	8
13.37	7

Cefalosporin, ciprofloxacin and Gentamycin has long been the known as the drug of choice for the treatment of typhoid fever in Sulaymani hospitals.

S. enterica is widely distributed in our environment and responsible for a wide range of clinical conditions some of them are fatal if untreated properly due to mistake in diagnosis specially typhoid fever and meningitis (Allen *et al*., 2003). The early administration of antibiotic treatment has proven to be highly effective in eliminating infections, but indiscriminate use of antibiotics due to incorrect identification has led to the emergence of multidrug-resistant strains of *S. enterica* serovar Typhi.

After the cases due to a lactose-fermenting isolates occurred in August-October 2009, there has been no additional clinical cases of typhoid fever in indoor or outdoor patient were recorded in Sulaymani city, and the Ceftraiaxone was the main antibiotic for controlling typhoid fever in Sulaymani city.

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