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 non-lactose fermenting (L-) *Salmonella enterica* serova *typhi* were isolated from blood of patients suffering from typhoid fever in indoor and outdoor of Sulaymani city-Iraq. The isolated *S. enterica* exhibited same characteristic as the type strain and L- *S. enterica* except for ONPG production. Serological examination revealed that all L+, L- and standerd strain possessed the antigenic formula as *S. enterica*. The appearance 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 a lactose 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.


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. enterica

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Sources of isolates</th>
<th>Oxidase</th>
<th>Kligler (slope)</th>
<th>Kligler (bottom)</th>
<th>H₂S production</th>
<th>Minitol fermentation</th>
<th>Acid formation</th>
<th>Lactose fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 L⁺ S. typhi</td>
<td>Blood</td>
<td>-</td>
<td>Red alkaline</td>
<td>Acid yellow</td>
<td>Little H₂S</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3 L⁻ S. typhi</td>
<td>Blood</td>
<td>-</td>
<td>Red alkaline</td>
<td>Acid yellow</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Standard strain</td>
<td>MCI</td>
<td>-</td>
<td>Red alkaline</td>
<td>Acid yellow</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+: 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 H₂S production and concurrently for lysine decarboxylase activity. Screening for H₂S 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, Ciprofloxac 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 non-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 ONPG test and the API 20E system. The L⁻ isolates show an acid butt and alkaline slant on kligler (KI) agar with small quantity of H₂S 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 20 E 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 H₂S when grow on KI agar Table 1.

Serological examination revealed that all lactose fermenters, three non 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 L⁺ 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
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; Lucaï 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) Cefalosporin, ciprofloxacin and Gentamycin has long been 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 Ceftraiazone was the main antibiotic for controlling typhoid fever in Sulaymani city.

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REFERENCES


