Journal of Microbiology and Infectious Diseases / JMID
2014; 4 (1): 30-32
doi: 10.5799/ahinjs.02.2014.01.0120

CASE REPORT

Lactose fermenting *Salmonella* Paratyphi A: A case report

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ABSTRACT

There are more than 2500 antigenic types of the genus Salmonella. Typhoid fever is caused by *Salmonella* Typhi, *Salmonella* Paratyphi A, B and C. Characteristically all four of these are non-fermenters of lactose. We report a case of lactose fermenting *Salmonella* Paratyphi A isolated from the blood sample of a 27 years old male with 12 days history of fever. Lactose fermenting colonies were isolated on Crystal MacConkey agar plate after 24 hours incubation at 35°C. It was identified using colony morphology, Gram staining, Oxidase test, sugar sets (Indole, Urea, Citrate, Triple sugar iron tests) and API 20E. It was confirmed as *Salmonella* Paratyphi A after positive agglutination reaction with *Salmonella* antisera polyvalent A-S and antiserum O_2.

The emergence of lactose fermenting strain of *Salmonella* Paratyphi A is important because it is likely to be missed or misdiagnosed. As *Salmonella* Paratyphi A is taking over *Salmonella* Typhi as cause of typhoid and is exhibiting high levels of drug resistance too. So in order to avoid incorrect identification it should be kept in mind that *S.* Paratyphi A can very rarely yield lactose fermenting colonies on Crystal MacConkey agar after 24 hours incubation. *J Microbiol Infect Dis 2014;4(1): 30-32*

Key words: Antibiotic resistance, Lactose fermentation, Salmonella, *Salmonella* Paratyphi A

Laktozu fermente eden *Salmonella* Paratyphi A: Bir vaka sunumu

ÖZET

Salmonella genusunda 2500'den fazla antijenik tipleri bulunmaktadır. *Salmonella* Typhi ve *Salmonella* Paratyphi A, B ve C tifo etkenidirler. Karakteristik olarak bu dört tür de laktozu fermente etmezler. Burada, 12 günlük ateş hikâyesi olan 27 yaşında bir erkek hastanın kanından izole edilen ve laktozu fermente eden bir *S.* Paratyphi A olgusu sunuldu. Laktozu fermente eden koloniler Crystal Mac Conkey agar besiyerinde 35°C'de 24 saatlik inkübasyon sonunda izole edildi. Bakteri, koloni morfolojisi, Gram boyama, Oksidaz testi, şeker testleri (indol, üre, sitrat, üç-şeker-demir) ve API 20E ile adlandırıldı. İzole edilen bakteri *Salmonella* polivalan A-S ve antiserum O_2 antiserumları ile pozitif aglütinasyon reaksiyonu vermesi ile teyit edildi.

Laktozu fermente eden *S.* Paratyphi A suşunun tanınması, atlanması veya yanlış tanı konması açısından önemlidir. S. Paratyphi A, tifo etkeni olarak S. Typhi'nin yerini almaktadır ve yüksek ilaç direncine sahiptir. Bu nedenle yanlış tanıdan kaçınmak için, S. Paratyphi A'nın nadiren 24 saatlik Crystal MacConkey agar üzerindeki inkübasyonunda laktozu fermente eden koloniler şeklinde üreyebileceği unutulmamalıdır.

Anahtar kelimeler: Antibiyotik direnci, Laktoz fermantasyonu, *Salmonella*, *Salmonella* Paratyphi A

INTRODUCTION

Salmonellae are Gram negative rods of the family Enterobacteriaceae. They are classified into two species; *Salmonella enterica* and *Salmonella bongori*[1] with *Salmonella enterica* further sub divided into 6 sub species. Most serotypes that are isolated in humans (>99.5%) belong to the sub species *S.* Enterica.[2] There are over 2500 antigenic types of the genus *Salmonella* which causes disease in humans.[3] and typhoid fever is caused by *S.* Typhi, *S.* Paratyphi A, B and C (also known as Typhoidal Salmonellae). Characteristically Salmonellae are non fermenters of lactose and this inability to ferment lactose is one of the important laboratory diagnostic criterions to distinguish them from other members of the same family.[2,4]

However some Salmonellae are known to ferment lactose and comprise less than 1% of the genus *Salmonella*.[2,5] They bear close similarity to the

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Received: 21.05.2013, Accepted: 27.11.2013

genus *Escherichia* and both are believed to diverge from the same ancestor.[6] However much genetic heterogeneity exists between the two, one such being the presence of *lac* operon in *Escherichia coli* which is absent in *S. enterica*. The *lac* operon is made of three genes; *lacZ, lacY* and *lacA* which are responsible for encoding β-galactosidase, lactose permease and transacetylase respectively. By acquiring this operon *Escherichia coli* can ferment lactose whereas *S. enterica* cannot due to the lack of *lac* operon. Nonetheless lactose fermenting strains reported in the genus *Salmonella* harbor genes responsible for lactose fermentation in extra chromosomal material like plasmids. These lactose fermenting strains may acquire the *lac* gene either by conjugation or transduction and they bear close similarity to the *lac* gene of *Escherichia coli*-K12.[7]

We report a case of lactose fermenting *S.* Paratyphi A isolated from the blood sample of a soldier admitted in a tertiary care hospital in January 2012.

CASE REPORT

A 27 years old male was admitted to the Military Hospital Rawalpindi with 12 days history of fever and headache. Fever was high grade, continuous and without rigors and chills. On abdominal examination, the spleen was palpable 3 cm below the left costal margin. Routine laboratory investigations were unremarkable.

His specimen of blood for culture was received in a bottle containing 50 ml Brain Heart Infusion broth. It was incubated at 35°C for 24 hours. Next day the sample was sub cultured on Blood and Crystal MacConkey agar and both these plates were incubated at 35°C for 24 hours.

After 24 hours incubation the blood agar plates showed grey white, circular, mucoid colonies with smooth convex surface, resembling those of Gram Negative Rods. The Crystal MacConkey agar plates yielded two types of colonies

1. Predominantly non lactose fermenting (NLF), Oxidase negative colonies.

2. A few 1-2 mm lactose fermenting colonies (LF).

Both the NLF and LF isolates were Catalase positive and were motile by hanging drop technique. They were dealt separately as per standard protocol i.e. after making a bacterial suspension equivalent to 0.5 McFarland's turbidity standard, conventional sugar sets were put up and antibiotic sensitivity using the modified Kirby-Bauer disc diffusion technique was done.

Next day identical reactions on Sugar Sets were observed for both the NLF as well as LF colonies i.e. both of them were Citrate, Indole and Urease negative while the Triple Sugar Iron showed an alkaline slant, slightly acidic butt with gas and no H_2S production. The purity plates yielded separate NLF and LF colonies as on Day1. The lactose fermenting colonies were also inoculated into API20E (Boimerieux) which yielded a score of 0104552 identifying the isolate to be *Salmonella* Paratyphi A. Following biochemical tests differentiated it from other motile, non H_2S producing Citrate negative members of family *Enterobacteriaceae* (on basis of API20E by Biomerieux & 8)

• *Escherichia coli* gives a positive Indole (90-95%), Lysine Decarboxylase (LDC-77%) and ONPG (95%) reactions which were all negative in our isolate.

• *Hafnia alvei* gives a positive LDC and Voges Proskauer(VP) reaction. VP was negative in our isolate as shown by API20E.

• Most of the *Yersinia* species gives a positive ONPG(70-80%) and Urease reaction (negative in our case).

• *Morganella morganii* will show a positive Urease and Indole reaction and will not ferment Mannitol (fermented by our isolate in API20E).

• Most of non H_2S producing strains of *Citrobacter* are ONPG positive (90%).

• *Edwardsiella tarda* produces H_2S in 94% strains but will also show a positive Indole and LDC reaction.

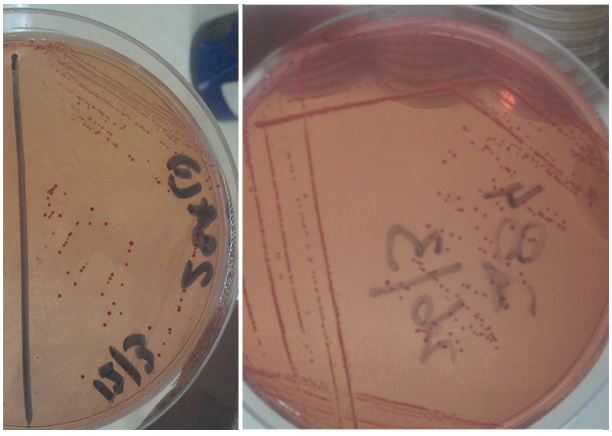

Figure 1. Lactose fermenting colonies on Crystal Mac Conkey agar after 24 hours incubation

Antibiotic sensitivity pattern of both the isolates (LF & NLF) was also identical i.e. they were sensitive to Ampicillin, Co-trimoxazole, Chloramphenicol and Ceftriaxone and were resistant to Ciprofloxacin and Azithromycin. Tough the zone of inhibition around the Ciprofloxacin disc was within sensitive

range but as per CLSI 2011 protocol it was reported to be resistant since the isolate showed resistance against Nalidixic acid (to avoid therapeutic failure).

Both the colonies showed positive agglutination reaction with *Salmonella* Antisera Polyvalent A-S (Denka Seiken Co Ltd.) and with antiserum O2 (Denka Seiken Co. Ltd). Therefore following the Kauffman-White classification for *Salmonella* diagnosis of *Salmonella* Paratyphi A was confirmed.[9]

DISCUSSION

Salmonella Paratyphi A is a non-lactose fermenting Typhoidal Salmonellae. Lactose fermenting strains of Salmonellae have been reported in case of *Salmonella typhimurium*, *Salmonella anatum*, *Salmonella tennessee, Salmonella newington* and *Salmonella seftenberg*[10-14] ; all of which are non Typhoidal Salmonellae. In case of Typhoidal Salmonellae only few cases of lactose fermenting strains of *Salmonella* Typhi has been reported.[4,15,16] To date no lactose fermenting *S.* Paratyphi A has been reported to the best of our knowledge.

Although some mutant Salmonellae can ferment sugars i.e. they can show acid production in a proportion of sugar peptone water cultures after incubation for two or more days. So they can be misread as fermenters unless a definitive reading time of 24 hours is adhered to.[2] However our isolate shows lactose fermentation after 24 hours and turns pale after 48 hours. This was the unusual characteristic of our isolate that is on Crystal MacConkey agar it yielded two types of colonies; one LF and the other NLF but both these types gave identical biochemical reactions (on TSI and API20E), antibiotic sensitivity patterns and agglutination reactions. The expiry date of Crystal Mac Conkey agar (Oxoid Code CM0115) as well as its positive and negative quality controls was also rechecked. For further confirmation the isolate was sub cultured onto plain MacConkey agar (Oxoid Code CM0007) and it showed similar growth pattern, yielding both LF and NLF colonies after 24 hour incubation at 35°C. The negative ONPG reaction in API20E given by the lactose fermenting isolate can be attributed to the production of lactobionic acid from oxidation of lactose - a rare property of certain Gram Negative bacteria.[17]

The emergence of lactose fermenting strain of *Salmonella* Paratyphi A is important because it is likely to be missed or misdiagnosed. As *Salmonella* Paratyphi A is taking over *Salmonella* Typhi as cause of typhoid fever and is exhibiting high levels of antibiotic resistance too. So in order to avoid incorrect identification it should be kept in mind that *S.* Paratyphi A can very rarely yield lactose fermenting colonies on Crystal MacConkey agar after 24 hours incubation.

Acknowledgement: No sponsors of study or conflicts of interests involved.

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