CASE SERIES

Human Salmonellosis Caused by Rare *Salmonella* Serotypes- Report of Two Cases

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Abstract:

Salmonellosis is a global disease caused by bacteria belonging to the genus *Salmonella*. It is the most important food-borne pathogen which is transmitted usually through contaminated food and water and is responsible for illnesses, hospitalisations and deaths worldwide. We present two cases of Salmonellosis caused by two unusual serotypes of *Salmonella*. Also, highlighted is the role of automation in bacterial identification and significance of serotyping of the bacterium.

Keywords: Automation, Gallinarum, *Salmonella*, Serotyping

Introduction:

The bacteria of genus *Salmonella* are spread extensively throughout the world and are troublesome in humans and animals leading to enteric fever, gastroenteritis and septicaemia. The genus is divided into two species, *Salmonella enterica* and *Salmonella bongori. S. enterica* is further divided into six sub-species which are *enterica, salamae, arizonae*, *diarizonae*, *houtenae* and *indica.* All these have several serovars or serotypes and more than 2500 serotypes have been identified [1]. *S. enterica* serovars Typhimurium and Enteridis are commonly responsible in developed countries. But it is reported that in other regions, other serovars are more prevalent [2]. Change in prevalence of specific serovars or presence of a new serovar can be the result of movements of people, animals and food [2].We present two cases of Salmonellosis caused by two different and rare serotypes. These were identified differently by the automated system and serotyping method. Hence, also focussed here is the fact that the use of automation for bacterial identification can prove helpful up to certain extent beyond which comes the role of serotyping. Correct serotyping helps to identify a new or an unusual serotype [2].

Case Report One:

Eight year old male child presented with high grade fever since seven days. There was no history of nausea, vomiting, loose motions or other complaints. He was not vaccinated for typhoid. He did neither have pets at home nor did he or his family members have contact with chicken or poultry animals. He is vegetarian but consumes eggs occasionally. He resides along with his family in the hospital campus. He had travelled to another state fifteen days before this episode and had eaten roadside food. Examination did not reveal significant findings. On admission, his complete blood count was 5,900 and Widal test showed an antibody titre of 1:160 to both *S. typhi* 'H' and 'O' antigens. Other blood investigations were normal. Blood culture was positive which showed Gram negative bacilli. On subculture, it grew grey mucoid colonies on Blood agar, non-lactose fermenting colonies on MacConkey agar (Fig. 1)

and red colonies with black centre on Xylose Lysine Deoxycholate (XLD) agar (Fig. 2) aerobically at 37˚C at the end of 24 hours. These appeared as Gram negative motile bacilli and were negative for oxidase, indole, urea, citrate and nitrate reduction test and triple sugar iron agar showed acid slant and alkaline butt (K/A) with no gas but H_2S in fair amount. Based on these conventional biochemical tests, it was identified as belonging to genus *Salmonella*. Agglutination with Salmonella polyvalent antisera was not done as it was not available. Identification and antibiotic susceptibility was done on VITEK2 Compact system using GNID and GN280 cards respectively. It was identified as *Salmonella* serovar Gallinarum by the system. Antibiotic susceptibility showed that isolate was sensitive to all antibiotics except trimethoprim / sulfamethoxazole. Also, it was resistant to azithromycin which was tested conventionally. The patient was given amoxycillin/ clavulanic acid after which he improved clinically and antibody titre was decreased in Widal test. Eventually, the isolate was sent to National *Salmonella* and *Escherichia* Centre at the Central Research Institute, Kasauli, for serotyping and the serotype was identified as Salmonella Jaffna (II), antigenic structure 9,12: d: z39.

Fig. 1: Non-lactose Fermenting Colonies of *Salmonella gallinarum* on MacConkey Agar

Fig. 2: Colonies of *Salmonella gallinarum* on XLD Agar

Case Report Two:

A month after the first case, 41 year old, vegetarian mentally retarded male, accompanied by his mother, presented with loose stools, abdominal pain, vomiting and giddiness since a week and fever of moderate grade since past three days. There was no history of having pets or contact with animals. He does not reside in hospital campus. He had travelled to another state and had also consumed food made at a religious gathering few days before this episode. He was not vaccinated for typhoid. On examination, epigastric tenderness was present. Complete blood count was 4,200. Widal test was negative. Other investigations were normal. Blood culture was positive which showed Gram negative bacilli. On subculture, it grew grey mucoid colonies on Blood agar, non-lactose fermenting colonies on MacConkey agar (Fig. 1) and red colonies with black centre on XLD agar (Fig. 2) aerobically at 37˚C at the end of 24 hours. These appeared as Gram negative motile bacilli which were negative for oxidase, indole, urea, citrate and nitrate reduction test and triple sugar iron agar showed acid slant and alkaline butt(K/A) with no gas but H2S was formed in fair amount.

It was identified as belonging to genus *Salmonella* based on manual biochemical tests. Agglutination

with polyvalent antisera was not done as it was not available. Identification and antibiotic susceptibility was done on VITEK2 Compact system using GNID and GN 280 cards respectively. It was identified as *Salmonella* serovar Gallinarum by the system. Antibiotic susceptibility showed the isolate to be sensitive to all antibiotics except trimethoprim / sulfamethoxazole. He was treated with intravenous ceftriaxone to which he responded. Eventually, the isolate was sent to National *Salmonella* and *Escherichia* Centre at the Central Research Institute, Kasauli, for serotyping and the serotype was identified as Salmonella Mathura, antigenic structure 9:i:enz 15.

Discussion:

Salmonellosis in humans occurs mostly through consumption of contaminated food products like pork, beef, poultry meat, eggs, vegetables, juices and other kinds of food. Occasionally, it may occur due to contaminated hands or contact of humans with infected pet animals [3]. Both of our patients had history of eating outside food few days before the current illness. The first patient also had history of occasional consumption of eggs. Both isolates were motile. Out of the many serotypes identified, Gallinarum is the only one that is nonmotile as it is obligatory nonflagellate [4, 5]. Both isolates also produced H_2S in fair amount in triple sugar iron test. Hence, on the basis of these and other findings on conventional biochemical tests, it was identified as *Salmonella species* [6]. Species identification was done using VITEK2 Compact system by GNID card. The organism was identified as *Salmonella* serovar Gallinarum by the system. According to this system, it fermented maltose and glucose and did not decarboxylate ornithine on the basis of which it was identified as serovar Gallinarum [5, 6]. However, S. gallinarum is host specific and causes fowl typhoid primarily infecting chickens and turkeys but other birds like pheasants, ducks, quail, guinea-fowl and peafowl are also at risk [3, 5, 6]. Due to its high adaptation to the host species, it is not of much public health importance [5]. Also, it does not colonise the alimentary tract well in these hosts in the absence of clinical disease and hence hardly ever enters the food chain. So, it is not known to cause human infection [7]. The isolate was sent for serotyping which helps to identify unusual serotypes or change in the presence of existing ones [2]. Serotyping revealed the first serotype as Jaffna and the second one as Mathura. A study by Basu *et al.* showed that serotype Jaffna was isolated from Indians many years back [8]. Due to increasing world population, there is increase production in large scale farms to meet the increasing demand for food. As a result, *Salmonella* serovars which have nonspecific hosts disseminate easily among animals once introduced in farms and are difficult to eradicate from farms and products used as food or for preparing it [3]. Thus there is a strong link between human Salmonellosis and animal food which is strengthened when people, animals and food move from one place to another [2]. Both our patients had history of travelling to another state prior to this illness. The global distribution of Salmonella serovars has changed over the years from *S. enteritidis* and *S. typhimurium to S. infantis* and *S. agona*. In Asia, *S. virchow* was quiet common earlier. But the data on prevalence of serovars is very limited. Also, their global distribution in humans is complex due to multiple

factors involved [10]. So, it is possible that our patients may have acquired these serotypes which were prevalent years back. In Vitek2 Compact system, test data from an unknown organism are compared to the respective database of the system which is formed with large strain sets of well-characterised organisms tested under various culture conditions. An unknown biopattern is compared to this database of reactions and a probability of identification is calculated [9].Nevertheless, clinical correlation and source of the isolate need to be taken into account. So, further confirmation in these cases was mandatory. Other methods like ribotyping and polymerase chain reaction for identification of *S. gallinarum* [5] were not available with us. In the first patient, antibody titres to Salmonella typhi antigens were raised on admission which decreased two days later. This could be result of antibiotics or an amnestic response in which rise in titre is only transient [1]. Serotyping was helpful in these cases to highlight the disremembered serotypes.

Conclusion:

In these cases, identification of the isolates by automation as a non-human and host specific serotype raised the alarm and demanded confirmation which led to these being confirmed as those serotypes which are consigned to oblivion but the possibility of their occurrence, especially in the developing world should be remembered.

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