Abstract

Gastroenteritis in human by *Salmonella enterica* subspecies *diarizonae* is rare and usually observed in neonates and children with history of contact with reptiles. *Salmonella enterica* subspecies *diarizonae* were isolated from two young adult male patients with symptoms of acute gastroenteritis. The isolates were identified by Vitek 2 compact system and agglutination by specific antisera. The isolates were susceptible to Amikacin, Imipenam, Cefazolin and Ceftazidime. Patient rapidly responded to Amikacin and Ceftazidime therapy. Arizona group *Salmonella should be looked in cases of gastroenteritis whenever there is history of proximity with reptiles.*

Introduction

Food borne diseases including, food borne intoxication and infections, are terms applied to illness acquired through the consumption of contaminated food; they are frequently and inaccurately referred to as food poisoning. The incidence becomes an outbreak only if the illness occurs within a short but variable period of time after a meal during which individuals have consumed foods in common [1]. Acute gastroenteritis caused by non-typhoidal *Salmonella* continues to be an important public health problem worldwide. Consumption of raw and undercooked meat, poultry eggs, and dairy products are the most common reasons for its transmission [2]. Reptile-associated salmonellosis is an emerging zoonosis in humans who handle reptiles directly or indirectly, causing mainly enteric illness. Rare cases of gastroenteritis in humans with *Salmonella enterica* subspecies *arizonae* [3] and *Salmonella enterica* subspecies *diarizonae* [4] are reported. Here we describe two cases of *Salmonella enterica* subspecies *diarizonae* gastroenteritis during a clinically suspected outbreak of gastroenteritis which occurred in a camp at Barmer district of Thar desert.

Cases

Four patients from a camp located in Gadra (Barmer) were presented on 9th March 2017 at Government Hospital, Barmer (Rajasthan) with sudden onset of diarrhea with or without abdominal pain and vomiting. Later 12 more patients from same camp were admitted on 10th March. The patients had a history of watery diarrhea with 10–12 motions/day and vomiting 2–5 times/day in the past 2 days. Their skin and mouth appeared dry. All the patients were male, and their age ranged between 18 and 45 years. Complete Blood Counts of all the patients were performed and found within normal range. Stool samples were sent to Microbiology Department of Dr Sampurnanand Medical College, Jodhpur on 11th March 2017. No leftover food sample was sent for testing. All the stool samples were semi-formed in consistency with no pus, blood or worm in them. Stool samples were inoculated on MacConkey agar, Alkaline Phosphate Water (APW) and Selanite F broth. Subcultures were made on Thiosulfate citrate bile salts sucrose (TCBS) agar from APW and on Xylose lysine deoxycholate (XLD) agar from Selanite F broth after 8 hours of incubation.

Gram staining, Hanging drop motility test, Saline and Iodine mount of stool samples were also performed. Few Gram-negative bacilli were observed in Gram staining. Hanging drop motility revealed the bacteria as actively motile but the motility was not suggestive for *Vibrio* species. No RBCs, trophozoite, ova or cyst was observed in saline and iodine mounts. Pus cells were found in all the stool samples but were abundantly present in four samples.

No intestinal pathogen grown in 14 samples however in 2 samples non-lactose fermenting colonies (NLF) grew on...
MacConkey agar and red colonies with black centers grew in XLD medium. Both the growths of NLF colonies were further subjected to Gram staining and routine biochemical testing (triple sugar iron agar, indole, methyl red, voses proskeur, citrate, mannitol, urea and motility). Both organisms were motile gram-negative bacilli but the results were inconclusive. Further biotyping was performed which identified the organisms as Salmonella enterica. The isolates were later subjected for identification and antibiotic susceptibility testing in Vitek® 2 compact (bioMérieux, Marcy-l’Étoile, France).

Both isolates were identified as Salmonella enterica subspecies diarizonae with similar antiobigram pattern. They were sensitive to Amikacin, Imipenem, Cefazolin and Ceftazidime. Augmentin, Aztreonam, Ciprofloxacin and Nalidixic acid were found to be resistant. To confirm the identification, subcultures of both colonies were made on nutrient agar and incubated for 24 hours at 37°C. Fresh growths were tested for agglutination by specific antisera (Remel Europe Ltd, Dartford, UK).

Treatment with Amikacin and Ceftazidime was started and the patients improved within 4-5 days. As the isolated pathogens are associated with reptiles, the history of contact or contamination by reptiles was looked for. The camp is located in the desert region with dry and hot climate. These areas are filled with reptiles such as snakes and lizards but no confirmed history of any contact with these reptiles was made.

**Discussion**

In 1939, Caldwell and Ryerson isolated Arizona group organism for the first time from reptiles in America. This organism has been called by various names such as Salmonella arizonae, Paracolobacterium arizonae, Arizona arizonae, Arizona hinshawii, etc. [5] S. enterica ssp. diarizonae is part of the normal reptile intestinal flora but can cause disease in monotremes, turkeys, chickens, goats, and rarely in humans [6]. Salmonella enterica ssp diarizonae and arizonae are the major reptile-associated pathogens in humans [7] and are highly prevalent in free-living and captive reptiles, particularly in snakes [8,9]. S. enterica ssp. diarizonae enteritis or systemic infections have been well described in patients residing in the southern states of the USA and Europe. It is much rare, with only a few cases reported in the literature [10-13]. It can be difficult to identify S. enterica ssp. diarizonae due to their distinguishing biochemical features such as the ability to utilize malonate, liquefy gelatin and the inability to grow in the presence of KCN (potassium cyanide), which are generally not looked into for routine identification of bacterial isolates. Isolation of S. enterica ssp. diarizonae from the stools is difficult as some strains ferment lactose within 48 hours (approximately 15%) and they may be routinely discarded as non-pathogens. However, the presence of hydrogen sulfide is an important diagnostic clue during routine screening [14]. Most cases of invasive S. enterica ssp. diarizonae infection have been either in younger patients specially neonates or those with underlying diseases including collagen vascular diseases, malignancy, organ transplantation and HIV infection [4,5,15,16]. However, in this case both the patients were adults with no underlying diseases. The stool specimens were sent to Microbiology two days after the onset of symptoms which may be reason for pathogens not growing in most of the specimens. The leftover food was also not available to trace the source of infection. The camp is present in Thar Desert region which is largely inhabited by reptiles such as lizards and snakes. Snakes and lizards frequently break into the compound area of the camp. Only the history of snakes and lizards contaminating the camp. Only the history of snakes and lizards was made. The leftover food was also not available to trace the source of infection. The camp is present in Thar Desert region which is largely inhabited by reptiles such as lizards and snakes. Snakes and lizards frequently break into the compound area of the camp. Only the history of snakes and lizards was made.

**Conclusion**

Incidences of Arizona group of Salmonella are rare but exist in India. S. enterica ssp. diarizonae can cause gastroenteritis in healthy adults. The laboratory should also attempt to detect Arizona group in patients with gastroenteritis and have history with proximity to reptiles.

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