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Case Report

Extended Spectrum Beta-lactamase producing *Salmonella Lindenberg* gastroenteritis

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Abstract

Introduction: Increasing antimicrobial resistance among non-typhoidal *Salmonella* (NTS) is a major public health issue especially in developing countries and is partly due to the use of antimicrobials in animal feeds as growth promoters. NTS are often associated with self-limiting acute gastroenteritis (AGE). Nevertheless, fluoroquinolones and third-generation cephalosporins are currently used in the treatment of severe diarrhoeal infections.

Methodology: We report the case of a 30-year-old male who presented with clinical symptoms of moderate gastroenteritis. Stool culture and antibiotic susceptibility was performed as per standard microbiological methods. Molecular detection of *bla* genes was carried out by PCR.

Results: The isolate was confirmed as *S. Lindenberg* by serotyping. The isolate exhibited dual resistance to fluoroquinolone and third generation cephalosporins. The isolate was an ESBL producer and harboured bla_{SHV}. Based on the antibiotic susceptibility pattern, the patient was successfully treated with ceftriaxone-tazobactam.

Conclusion: Presently, there are no Indian reports on the bla_{SHV} positive ESBL producing *S. Lindenberg* gastroenteritis. We report on the successful management of the first case of acute gastroenteritis caused by *S. Lindenberg* that exhibited dual resistance to fluoroquinolone and third generation cephalosporins. Continued surveillance of the antibiotic resistance pattern of the Non-typhoidal *Salmonella* serovars circulating in the geographical region is warranted.

Key words: Acute gastroenteritis; AGE; ESBL; fluoroquinolone; *Salmonella; S. Lindenberg*.

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Introduction

Non-typhoidal *Salmonella* (NTS) serovars of the species *enterica* are the most common cause of foodborne salmonellosis and represent a major public health concern. NTS are often associated with self-limiting acute gastroenteritis (AGE) and are primarily transmitted by the consumption of contaminated food products of animal origin especially poultry, meat and eggs containing as few as 10^3 bacteria [1]. The estimated annual burden of NTS is high with the best data coming from South East Asia with a reported 22,805,000 illnesses, 37,600 deaths and an incidence per 1000 person– years of 3980 [2]. In our country, very few studies have reported the incidence of severe NTS infection but there is evidence that invasive disease is an important cause of hospital admissions in southern India, other systemic manifestations and its associated deaths [3-6].

Routine laboratory diagnosis of NTS gastroenteritis rely on the isolation of the organism by stool culture in selective media, followed by biochemical characterization and serotyping with commercially available polyvalent antisera. Though genotyping by molecular methods are available to reliably distinguish the members within a serogroup, lack of standardization, time and reagent cost limits the use of typing techniques in diagnostic clinical laboratories [7]. Whole genome sequencing is currently used as the sole, alternative method for serotype discrimination [8] but in low to middle income country settings, the isolates are generally shipped to the reference laboratories for complete serotyping. Serotyping is a skilled technique and the differentiation of closely related serotypes can take several weeks.

Case Presentation

A 30-year-old male presented to the Salem steel plant hospital, Salem, South India with history of loose stools since 2 days- 10 episodes/day, vomiting-once/day, abdominal colic, mild dehydration and low-grade fever (99.4°F). CVS: S1 S2 +, RS: Clear, Chest-no tenderness, BP: 130/84, PR: 116/minute. Ultrasound abdomen: normal study. Results from haematological tests revealed the following values: hemoglobin- 14.6 g dL^{-1}, Total Count – 10,500 cells/µL, Platelet- 1.76 lakhs, Random blood glucose- 86 mg/dL, Urea-28 mg/dL, Creatinine-1.0 mg/dL. Serum electrolytes: Ca-9.5 $mEqL^{-1}$, Na-134 $mEqL^{-1}$, K-35 $mEqL^{-1}$, Cl-104 $mEqL^{-1}$, HCO_3-26 $mEqL^{-1}$.

Routine stool examination revealed the following, mucus 2+, Pus cells 4+, RBCs 2+, Occult blood-negative, parasitic Ova/cysts-nil. Stool culture by standard microbiological methods yielded non-lactose fermenting gram negative bacilli. The isolate was provisionally identified as *Salmonella spp* by the standard biochemical tests. The isolate was sent to Central research institute, Kasauli, Himachal Pradesh, India for serotyping, where it was identified as *Salmonella enterica* Serovar *Lindenberg* with the antigenic structure (6,8:i:1,2).

Antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion method as per CLSI guidelines [9]. The isolate was susceptible to chloramphenicol and co-trimoxazole. However, it was resistant to Ampicillin, ciprofloxacin, pefloxacin, tetracycline, ceftazidime, ceftriaxone and intermediate susceptibility was observed for cefotaxime. Nevertheless, the isolate was susceptible to ceftriaxone-tazobactam. The isolate was also screened for carbapenamase production using ertapenem (10µg), meropenem (10µg), imipenem (10µg) and doripenem (10µg) discs (HiMedia Laboratories Pvt Ltd, Mumbai, India) and was found to be a non-carbapenemase producer i.e. susceptible to all the 4 carbapenems tested.

Extended spectrum beta-lactamases (ESBL) production was assessed by combined disc test (ceftazidime (CAZ: 30µg): ceftazidime-clavulanic acid (CAC: 30µg/10µg) and Cefotaxime (CTX: 30µg): cefotaxime-clavulanic acid (CEC: 30µg/10µg) (Himedia Laboratories Pvt Ltd, Mumbai, India). *Klebsiella pneumoniae* ATCC 700603 was included as the control. The test isolate exhibited an increment in zone diameter when combined with clavulanic acid (CAZ:CAC: 3 mm, CTX: CEC: 9 mm) which confirmed the production of ESBL. The minimum inhibitory concentration (MIC) was determined using Ezy MIC™ strip (CTX/CTX+CA) & Ezy MIC™ strip (CAZ/CAC+CA) (Himedia Laboratories Pvt Ltd, Mumbai, India). The MIC of Cefotaxime, cefotaxime-clavulanic acid was >15µg/mL and >1µg/mL respectively, while that of ceftazidime; ceftazidime-clavulanic acid was >32µg/mL and >4µg/mL respectively indicating >3 twofold concentration decrease in MIC values when combined with clavulanate compared to MIC of the antibiotic tested alone. Further the presence of genes coding for beta-lactamases, $bla_{CTX\text{-}M\ group\ 1}$, $bla_{CTX\text{-}M,}$ bla_{SHV}, bla_{TEM} were analysed by PCR [10-11]. BAA2146 was included as the positive control for all the 3 *bla* genes. The isolate harbored bla_{SHV} gene nevertheless, $bla_{CTX\text{-}M\ group\ 1}$ and bla_{TEM} was not detected. The bla_{SHV} PCR product had been sequenced and the sequence has been deposited in GenBank under the accession number, MK816317.

The patient was treated with IV fluids, Rantac, Rifagut, Zedott, Emeset. The patient was initially put on ofloxacin-ornidazole. Based on the antibiotic susceptibility pattern ceftriaxone-tazobactam was started. The patient responded to therapy. He was afebrile, BP: 102/70, PR: 68/min, loose stools- 2 episodes/day, vomiting- once/day, hydration-good and was discharged on the 3rd day.

Discussion

In humans, the outcome of infection with *Salmonella* depends primarily on the infecting serovar and the host factors [12]. NTS cause self-limiting gastroenteritis in immunocompetent patients and invasive infections in immunocompromised individuals. Among NTS, the most common serovar associated with human salmonellosis is *S. enterica* serovar *enteritidis* and *S. enterica* serovar *Typhimurium* [5,12]. According to the AMR surveillance network, ICMR 2017, the isolation rate of the *Salmonella spp* (other than *S. Typhi*, *S. Paratyphi A* and *S. Typhimurium*) in faeces is reported to be very high (85.7%) compared to *S. Typhimurium* (14.3%) [13].

Previous Indian report [14] on *S. Lindenberg* had been from invasive infections, nevertheless, here we report, for the first time a case of *S. Lindenberg* gastroenteritis. *S. Lindenberg* differs from *S. Typhimurium* in having C2 serogroup O antigens, nevertheless they possess similar H antigens, i:1,2 could lead to the misreporting of the serovar. Fluoroquinolones and third-generation cephalosporins are currently used in the treatment of severe *Salmonella* infections, while antimicrobial therapy is not indicated for mild or moderate gastroenteritis. Here, we report a case of dual fluoroquinolone resistance and ESBL production exhibited by *S. Lindenberg* that carried

bla_{SHV} which is a matter of concern. This indicates that empirical treatment of AGE with ciprofloxacin /ceftriaxone could no longer be effective.

Increasing antimicrobial resistance among NTS isolates is a major public health issue and is possibly due to the use of antimicrobials in animal feeds as growth promoters. Combined ESBL and fluoroquinolone resistance among NTS has important clinical implications as this limits the antimicrobial treatment options. Hence, continued surveillance of the antibiotic resistance pattern of the NTS serovars circulating in the geographical region is warranted.

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