CASE REPORTS

EXTENDED SPECTRUM β- LACTAMASE PRODUCING *SHIGELLA FLEXNERI*- A CASE REPORT

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ABSTRACT

Infection by *Shigella* species is one of the major cause of morbidity especially in children with diarrhea. Extended spectrum β lactamases (ESBL) confer resistance to most of the beta-lactam antibiotics, including penicillins, cephalosporins and aztreonam. Its production in *Shigellae* is of concern especially in children, in whom treatment options are already limited. Third generation cephalosporins are used for treating diarrhea due to multidrug resistant *Shigella* species in children, but ESBL production renders them ineffective too. We report an extended spectrum β lactamase producing *Shigella flexneri*, isolated from the stool sample of a 3-year-old boy.

INTRODUCTION

Shigellosis, an important public health problem of children and elderly especially in the developing countries, is caused by *Shigella* species *(S. dysenteriae, S. flexneri, S. boydi and S. sonnei*). The diarrhea usually is self limiting in 2-3 days but antibiotics can shorten the course of the illness.

Extended spectrum beta lactamases (ESBLs) are an important mode of resistance in Gram negative bacteria but only a few have been reported in *Shigella* species[1]. ESBL producing organisms often also possess resistance determinants to other important antibiotic groups (eg. aminoglycosides and fluoroquinolones), leaving a limited range of effective antibiotics. Use of third generation cephalosporins is increasing especially in cases of multi-drug resistant *Shigella* infection in paediatric cases (where ciprofloxacin is used with caution due to the adverse effects) and in adults (when the organism is found resistant to fluoroquinolones). Extensive use of broad spectrum antibiotics has lead to the possible emergence of extended spectrum beta lactamase (ESBL) producing *Shigella* species[2]. We report a case of ESBL producing *Shigella flexneri* isolated from stool sample of a 3-year-old boy.

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CASE REPORT

Stool sample of a 3-year-old boy was received at the Department of Microbiology, Army Medical College from Military Hospital, Rawalpindi for culture & sensitivity testing. The boy presented in the pediatric ward with two days history of dysentery and high grade fever. The stools were slightly loose in consistency, occurring 2-3 times a day and mixed with blood.

On routine examination the stool was slightly red in color and had loose consistency. On microscopy 10-12 pus cells / HPF and numerous R.B.C. / HPF were seen. The stool sample was inoculated on MacConkey agar (Oxoid, UK) and xylose lysine decarboxylase agar (XLD) (Oxoid, UK). The plates were then incubated at 35°C for 24 hours. Non-lactose fermenting colonies (pale colored on MacConkey and pink colored colonies on XLD without black centers) were observed after incubation. Upon further testing they were identified as catalase positive, oxidase negative, non-motile Gram negative rods. A bacterial suspension equivalent to 0.5 McFarland turbidity standard was prepared and inoculated into API 20E (bioMerieux, France) for biochemical testing. The organism was identified as *Shigella flexneri* and

confirmed with *Shigella flexneri* antiserum (Wellcome diagnostics).

Antibiotic sensitivity of the organism was tested by using the modified Kirby-Bauer disk diffusion method. The organism was tested by applying discs of ampicillin(10µg), trimethoprim-sulphamethoxazole (25µg), ciprofloxacin (5µg) on Mueller-Hinton agar (Oxoid, UK). *Escherichia coli* ATCC 25922 were used as control strains. To check for ESBL production a sensitivity disk containing amoxicillin-clavulanate 20/10 µg (Oxoid) as the inhibitor of beta-lactamase was placed in the center of the Mueller-Hinton agar plate and ceftazidime 30µg, ceftriaxone 30 µg (Oxoid) and aztreonam 30 µg (Oxoid) disks were placed at 25 mm (center to center) from the amoxicillin-clavulanate disk.

ESBL production was confirmed by observing an enhancement of the zone of inhibition around ceftazidime, ceftriaxone and aztreonam in the presence of clavulanate. The isolate was sensitive to ciprofloxacin so the patient was advised ciprofloxacin and his symptoms improved i.e. was afebrile and stool consistency improved. He was discharged from the hospital on the 3rd day of treatment and was advised to complete the antibiotic course and after 1 week was asymptomatic on follow up visit.

DISCUSSION

Multidrug-resistance in *Shigellae* is a matter of growing concern all over the world, for which third-generation cephalosporins and fluoroquinolones are increasingly being used[3]. Extended spectrum beta lactamase production in *Shigellae* has lead to the development of resistance in Shigella to third-generation cephalosporins as well[4]. In *Shigella* however, ESBL production is rare worldwide[5].

In 2001, an Ambler class-A ESBL producing *S. flexneri* was isolated from the stool sample of a 16 month old Algerian boy from a hospital in France[6]. This isolate harboured a SHV-2 β lactamase gene on c. 80kb self-transferable plasmid. Previously, a SHV-11 ESBL-producing *S. dysenteriae* strain was first time reported in India in 1999[7].

In Pakistan the first third generation cephalosporin resistant *Shigella flexneri* was isolated in The Aga Khan University Hospital, Karachi, Pakistan, in 2005, but it was not ESBL producer[8]. A CTX-M type ESBL producing *Shigella sonnei* was isolated from a patient in United States who had recently traveled to

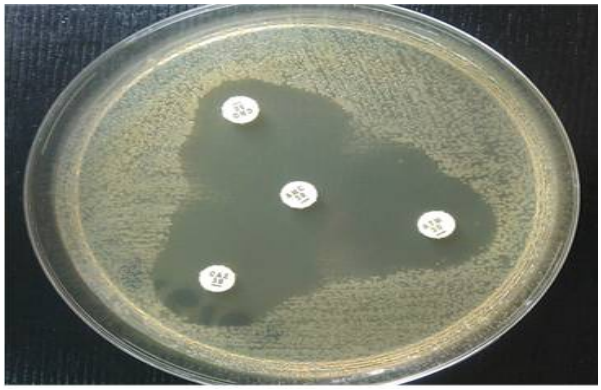

Figure: Demonstration of ESBL production by zone enhancement.

Pakistan[9]. In 2009 an ESBL producing *S. flexneri* was isolated in our department from an 8-year old girl suffering from dysentery[10]. A study to determine the trends in antimicrobial resistance in *Shigella* species in Karachi, Pakistan was conducted at the Aga Khan University Hospital, on stool samples collected between 1996 and 2007. Ceftriaxone resistance emerged in 2001 and increased to 8% in year 2007. All ceftriaxone resistant *Shigellae* were ESBL producers. Out of a total of 1573, 857(54.5%) were *S. flexneri* and of these 27 S. flexneri were ESBL producers[11].

Seven ESBL producing Shigella were reported from Japan[12]. In Iran 4 ESBL producers were found in children less than 12 years, of which 1 was *S. flexneri* and 3 were *S.sonnei*[13]. In a study done in India from 2001-2009, 119 *S. flexneri* were isolated, of these 20 were cefipime resistant but only 09 were ESBL producers[14].

CONCLUSION

Treatment guidelines for diarrhea should be formulated and monitored at the national level to

check the emerging resistance of organisms to broad spectrum antibiotics. Unnecessary and over-enthusiastic use of antibiotics for treatment of various infections should be avoided to prevent the spread of ESBL producing strains of *Shigella* as it may become a significant problem in near future.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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