Ctx & Tem Genes in Fecal Bacteria Isolated from Autism Spectrum Disorder Children

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ABSTRACT

31 cefotaxime resistant fecal isolates out of total 88 isolates from ASD children and control children subjected to antibiotic resistant. Performing PCR to detect blaCTX and blaTEM , blaCTX gene were nonsignificantly associated to ASD fecal isolates while blaTEM gene was 100% associated with fecal bacteria isolated from ASD .

Key Words: ASD, blaCTX, blaTEM

INTRODUCTION

The term autism spectrum disorder was used regularly to refer to group of common symptoms, but the presence and severity of these symptoms vary greatly. People with autism mainly suffer from social communication[1]. Available twin studies have shown that environmental factors are more important than genetic predisposition. Among these factors, microbial dysbiosis is of increasing interest, with more and more reports in animal models and human epidemiological studies linking destructive changes in the intestinal flora with ASD symptoms[2].

Bacteria in the GIT perform a variety of host symbiotic functions, from the digestion and production of bioactive metabolites to their effects on the healthy development and function of the immune system. All of these local effects on the GIT have the ability to affect the brain through neural connections[3].

The exponential incidence of antibiotic-resistant infections, especially those caused by Gram-negative pathogens, is a major public health concern[4]. Tolerance has been reported since the discovery of penicillin. With the advent of the massive use of antibiotics, appropriate or not, resistance has been continuously selected, both in commensal bacteria, zoonotic bacteria or pathogenic bacteria[5].

CTX-M β-lactamases are class A enzymes that are characterized by the ability to efficiently hydrolyze cefotaxime. These enzymes have spread globally to become the most widespread ESBLs in Gram-negative bacteria[6]. The TEM β-lactamases are among the best-studied antibiotic resistance enzymes around. They act by hydrolysing the b-lactam ring of penicillins, cephalosporins and related antibiotics and are found at high frequencies in hospitals and clinics around the world [7].

MATERIALS AND METHODS

STUDY GROUPS

This study was designed to determine the association of three parameters(serum IgA,C5a and serotonin) with gastrointestinal complications in ASD. A total of 88 children were included in the present study (50 ASD and 38 non-ASD control children). The studied groups were aged 3-16 years.

EXCLUDED GROUPS

Children with neurological disorders other than autism spectrum disorder (ASD) were excluded.

STUDY QUESTIONNAIRE

A questionnaire was designed to meet the objective of this study.

SAMPLE COLLECTION

Patients' attendants were provided with plastic container with leak-proof lid. Once the specimen has been placed in the container, the lid was sealed. They asked to deliver the specimen immediately to the laboratory after its collection[8].

BACTERIAL ISOLATION AND DIAGNOSIS

Stool samples were inoculated onto MacConkey agar. After overnight incubation at 37°C individual colonies were picked up from MacConkey agar and screened for their identities using VITEK.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The susceptibility of organisms to antimicrobial agents was determined by disk diffusion method. The antibiotics used were: Cefotaxime (30µg, Mastdiscs) , Imipenim (10 µg, Mastdiscs), Gentamicin (10 µg, Mastdiscs), Amikacin (30 µg, Mastdiscs), Doxycycycline (30 µg, Mastdiscs), Ciprofloxacin (5 µg, Mastdiscs), Nalidixic acid (30 µg, Mastdiscs), Nitrofurantoin (300 µg, Mastdiscs) and Chloramphenicol (30 µg, Mastdiscs).

DNA EXTRACTION AND GENE DETECTION

Bacterial DNA from cefotaxime resistant isolates were extracted using (ZYMO, USA) kit. The concentration of the extracted DNA

was determined using Quantus Fluorometer. The primers CTX-M-U1 (5`-ATGTGCAGYACCAGTAARGTKATGGC-3`) and CTX-M-U2(5`-ATGTGCAGYACCAGTAARGTKATGGC-3`) were used to detect the blaCTX-M gene, and the primers TEM-F(5`-CTT CCT GTT TTT GCT CAC CCA-3`) and TEM-R (5`-TAC GAT ACG GGA GGG CTT AC-3`) were used to detect blaTEM gene as described previously[9],[10]. The PCR products were visualized by 2% agarose gel electrophoresis staining with Ethidium bromide (10 mg/ml).See figure 1&2

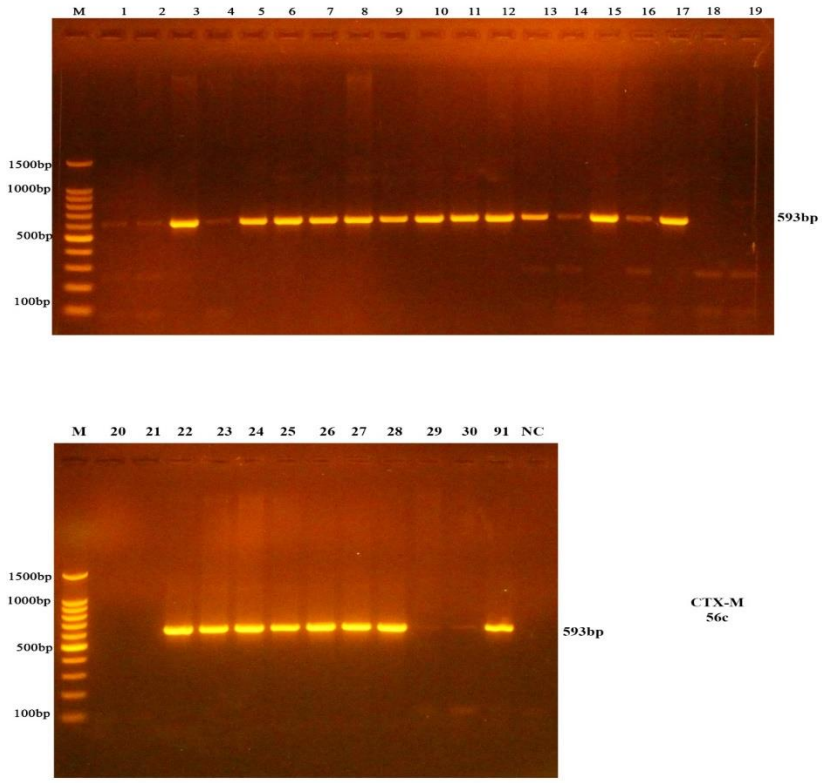

Figure 1 The amplification of *CTX* gene in bacterial samples fractionated on 1.5% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker

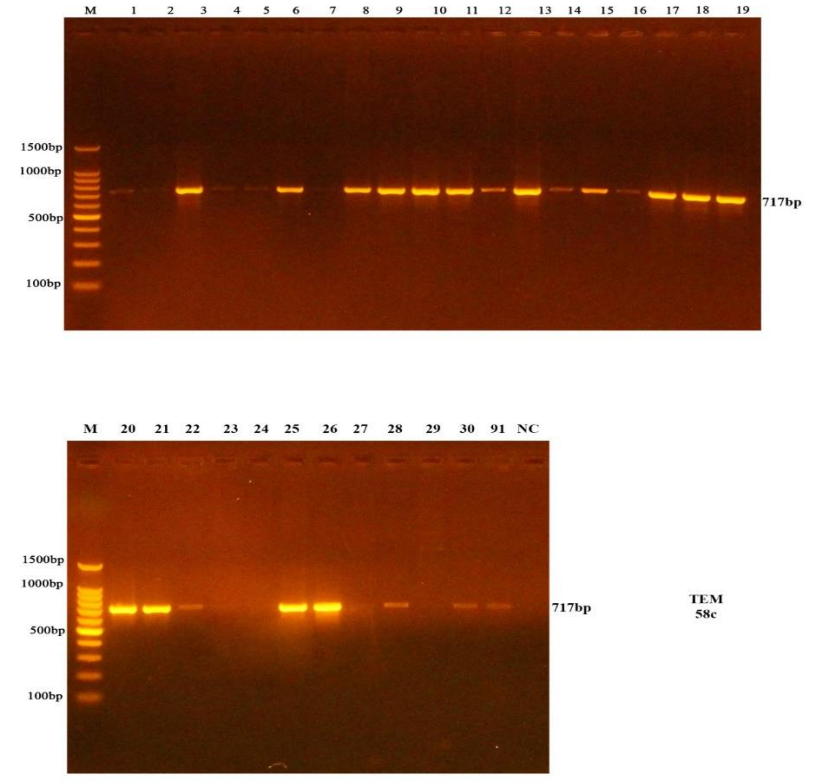

Figure 2 The amplification of *TEM* gene in bacterial samples fractionated on 1.5% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker

RESULTS AND DISCUSSION

Out of total 88 isolated bacteria, the phenotypically cefotaxime-resistant bacteria were 19 isolates(38%) from ASD (16 isolates *E.coli*, 1 isolate *K.pneumoniae*, 1 isolate *Sh.sonnie*, and 1 isolate *K.oxytoca*) and 12 isolates (31.5%) from control (all were *E.coli*).

Detection of *CTX* gene in phenotypically cefotaxime-resistant bacteria

14 isolates (73.6%) of the 19 phenotypically cefotaxime-resistant bacteria isolated from ASD in Figure (3) were carrying *CTX* gene whereas 7 isolates (58.3%) out of 12 cefotaxime-resistant bacteria in controls was carrying the mentioned gene. There was no significant difference (P=0.693).

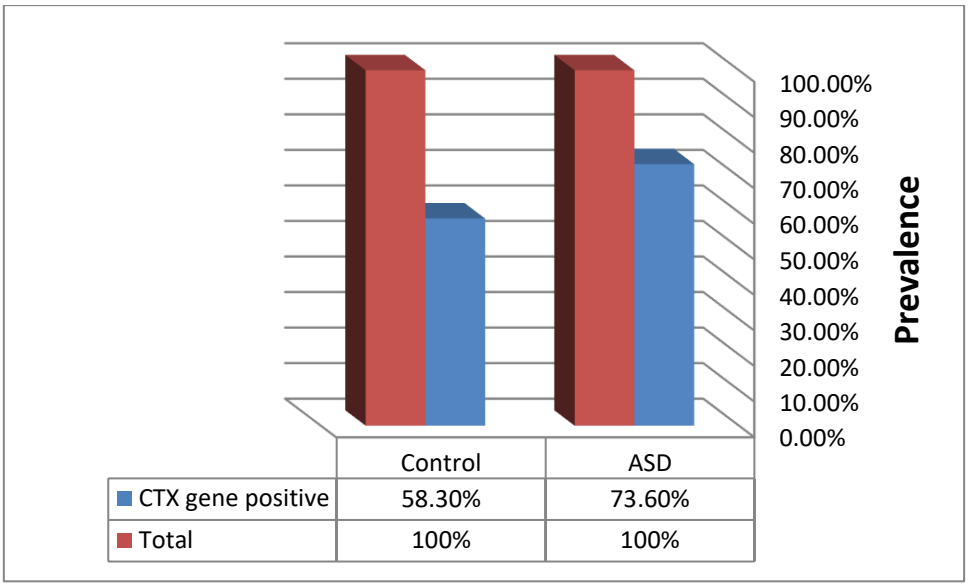

Figure (3) Prevalence of *CTX* gene-carrying phenotypical cefotaxime-resistant isolated bacteria

Detection of *TEM* gene in phenotypically cefotaxime-resistant bacteria

Comparative analysis showed statistically highly significant difference (P=0.0006) between phenotypically cefotaxime-resistant bacteria in the two groups (cases and controls) in carrying *TEM* gene where 19 isolate (100%) of ASD bacterial isolate carry *TEM* gene versus 8 isolates (66.6%) of those of control. See figure 4

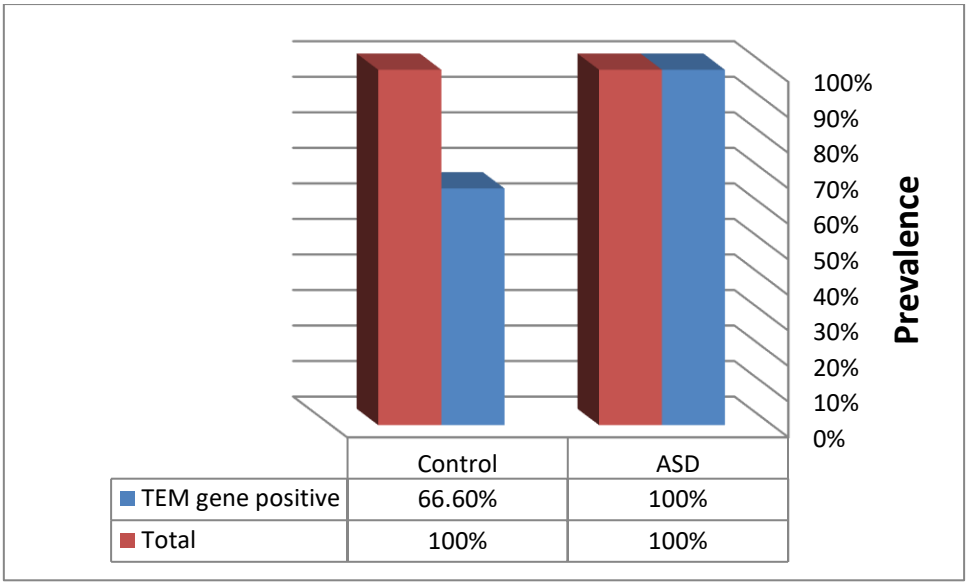

Figure (4) Prevalence of *TEM* gene-carrying phenotypically cefotaxime-resistant isolated bacteria.

The vast majority of ESBLs are encoded by $bla_{\text{CTX-M}}$, $bla_{\text{CTX-M-1}}$, bla_{SHV}, bla_{TEM} genes[11]. ESBL-producing Enterobacteriaceae are important member of antibiotic resistant bacteria that cause hospital and community acquired infections[12]. In a Thai study[13] the genetic analysis of studied isolates by PCR revealed that 94.3% were positive for the CTX-M genes and the majority of CTX-M ESBL producing bacteria were identified as *E.coli* (85.1%) followed by *Klebsiella spp* (5.7%) and *Citrobacter spp* (5.7%). Nepalese study[14]conducted on healthy adults result that the predominant type was *TEM* (92%) followed by *bla*CTX-M (60%).

CONCLUSION

blaTEM gene is highly associated with fecal bacteria isolated from ASD children.

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