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Original Article

Extended Spectrum Beta-Lactamase (ESBL) Producing Enterobacteria in Aquatic Environmental Sources of Bangladesh

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Extended-spectrum β-lactamase (ESBL)-producing Enterobacteria has become a considerable global concern because of their potential dissemination in humans, in domestic animals, wildlife and the environment. The present study aimed to explore the ESBL producing enterobacteria in aquatic sources of Bangladesh as the water may be the potential source of dissemination of this alarming antimicrobial resistance. A total of 94 water samples (53 tube well and 41 supplied water) were collected from 35 districts of Bangladesh from which 60 Enterobacter were isolated based on their biochemical profile. Among these 36 were *Enterobacter sp* and 24 were *E coli*. 73% of the isolates were Multidrug resistant (MDR) as revealed by Antimicrobial susceptibility test whereas 29% of these MDR isolates were phenotypically detected as ESBL producing as observed by Double disk synergy test (DDST) test. The ESBL type SHV was found to be dominant among the isolates revealed by PCR. Therefore, strategies can be employed to reduce the dissemination of ESBL producing bacteria in the aquatic sources which may threaten the human life, animal and surrounding environment.

Keywords: Extended-spectrum β-lactamase (ESBL), multidrug resistant, Enterobacteria, aquatic sources

Enterobacteriaceae with extended-spectrum beta lactamase (ESBL) enzymes are recognized as a major concern worldwide posing significant threat to human health and leading to healthcare expenditure (1, 2). These ESBL producing bacteria were first observed in Germany in the 1980s and are now reported worldwide both in industrialized and developing countries (1, 3). These situations are rapidly growing as a serious issue in developing countries like Bangladesh due to the frequent emergence of enteropathogens which cause life-threatening infections (4). ESBLs are enzymes that confer resistance to cephalosporins and mono-bactams by hydrolyzing their b-lactam ring, and evolved through series of amino acids substitutions (5, 6). Some ESBLs are derived from earlier, broad-spectrum beta-lactamases (e.g., the TEM, SHV and OXA families) whereas CTX-M, PER and KPC k-lactamases are now increasingly described (6, 7). The extended spectrum of activity of these enzymes is due to deviation from the parent enzyme by few point mutations (8). According to recent studies, CTX-M-15 b-lactamase is frequently found in humans and animals (9). CTX-M has become the most widespread class (10). ESBL genes have now been reported to be present in wastewater, surface water, sewage, and sediment samples (11, 12). In Bangladesh most studies have focused on

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clinical isolates to survey antimicrobial resistance, while the prevalence of ESBL producing bacteria in the environmental samples has not been so much explored. Therefore, the present study was a short endeavor to determine the prevalence of ESBL producing enteropathogenic bacteria in water from different parts of Bangladesh and to characterize the type of ESBL encoding genes they harbor.

Materials and methods

Sampling and isolation of *Enterobactriaceae* from the samples

A total of 94 samples (53 tube well and 41 supplied water) were collected within the period of October 2012 to August 2014 from 35 districts of Bangladesh. For Eneterobacteria isolation, samples were inoculated in nutrient agar plate. After an overnight incubation at 25 °C, several colonies were selected based on their distinguished morphology. From these, enterobacteria were screened after characteristic growth on MacConkey agar and Eosine methylene blue (EMB) agar; and conventional biochemical tests of sugar utilization mindole test, methyl red (MR) test, Voges-Proskauer (VP) test, citrate utilization test, moti-lity indole urease (MIU) assay, catalase and oxidase tests (13).

Antimicrobial Resistance profile of the isolates and phenotypic ESBL detection

The antimicrobial susceptibility of the test isolates was determined *in vitro* by using the standardized agar-disc-diffusion method known as the Kirbey-Bauer (14) which is a modification of Baur's method (15). Commercially available discs and Mueller-Hinton agar (Oxoid Limited, England) were used for the test. In total, 10 antibiotic disks were used in this study. From here the multidrug resistant isolate were selected for phenotypic screening of ESBL producing bacteria. All *Enterobacteriaceae* isolates that were resistant to one or more β-lactam antibiotics and also resistant to third generation cephalosporins, were subjected to double disk synergy test (DDST) for phenotypic screening of ESBL production (16). Ceftazidime (CAZ) (30 µg), cefotaxime (CTX), (30 µg) and amoxycillin / clavulanic acid, (AMC) (30 µg) discs were used.

Molecular characterization of the ESBL producing isolates

Plasmid DNA was extracted by using Birnboim dolly method, and was separated by horizontal electrophoresis. The molecular weight of the unknown plasmid DNA was determined on the basis of its mobility through agarose gel and was compared with the mobility of the known molecular weight plasmids of *Escherichia coli* V_{517}. Bacterial strains confirmed for producing ESBLs were further analyzed by PCR. Prior to PCR, DNA was extracted by boiling method. Representative strains showing a positive result for ESBLs were further analyzed for the presence of 4 common ESBL genes encoding TEM-, SHV-, CTX-M-, and OXA-type -lactamases, using PCR. 3 sets of specific primers TEM-F/TEM-R, SHV-F/SHV-R and CTX-MU1/CTX-MU2 were selected to screen these ESBL producing genes with respective reaction condition as described previously (17- 19). All PCR mixtures contained approximately 50 ng DNA templates, 1 × PCR buffer, 0.2 mM of each dNTPs, 0.2 mM of each primer and 1 U Taq DNA polymerase in 25 µl volume.

Results

Among the 94 water samples analyzed, 60 Enterobacteria were primary recovered from water samples according to the characteristic growth pattern on the selective media used in this study. They were further confirmed according to their biochemical profile such as IMViC, oxidase and catalase tests. *E coli* was isolated from 35% of the samples whereas 18% of the samples were revealed with the presence of *Enterobacter sp*. A total of 36 *E coli* and 24 *Enterobacter sp* were isolated from 94 water samples characterized in the present study.

Of 60 isolates tested, more than 26 % were resistant to at least one antibiotic out of 10 antibiotics tested. More than 73% of the isolates

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were resistant to 3 or more classes of antibiotics and were therefore defined as multi-drug resistant (MDR). From these, 18 were *E. coli* and 26 were *Enterobacter sp*. The most common resistant phenotype of the water isolates was to amoxicillin (AML) and Aztreonam (ATM). 13 of 44 MDR isolates were phenotypically screened as ESBL producing isolates according to the DDST method, indicating that a significant proportion (29%) of these isolates were ESBL producer.

Molecular characterization was executed employing three specific primers for bla $_{CTX}$, bla $_{TEM}$ and bla $_{SHV}$ .The obtained results revealed the presence of ESBL producing genes in 7 out of the 13 phenotypically ESBL producing isolates. The most frequent genotype was bla $_{SHV}$ (85%) followed by bla $_{ctx}$ (28%). .Bla $_{TEM}$ was not found in any of the isolates.

Discussion

The presence of isolates reported in the present study, indicates the fecal contamination of the drinking water which may be hazardous to the human health, causing enteric diseases. It becomes more potentially threat when these isolates from drinking water exhibit resistance to different antibiotics. Previous studies carried out in different countries demonstrated the presence of antibiotic resistant enterobacteria in pond, river, lakes and drinking water (20). Even in the drinking water of Bangladesh, the antibiotic resistant *E coli* was reported recently.

The residual effect of the extensively used antibiotics in human population and food chain may have been exerted selective pressure in the environment. The ESBL producing isolates were also resistant to different groups of antibiotics such as amoxicillin, ciprofloxacin, tetracycline, etc. So, the co-selection of different groups of antibiotics may exert the selective pressure which may contribute to the spread of these isolates (21).

This finding is utterly different from another study carried out in Bangladesh (2). But similarity was found with other studies (22, 23) indicating the prevalence of bla $_{SHV}$ gene. Plasmid profile analysis revealed the clonal diversity among these isolates as all of them were imparted with different patterns including one which contained no plasmid. Whether these plasmids encode the ESBL producing genes can be concluded by further conjugation experiments.

The present investigation was an endeavor to observe the prevalence of ESBL producing enterobacteria in drinking water of different areas of Bangladesh. As the samples were not collected from all of 64 districts, it may not represent the condition in entire country, but a large part of population live on this area. Although people are advised to consume the pre-treatment water, but this is not maintained. So, the presence of these MDR and ESBL producing Enterobacteria in the water may have a serious implication in the public health. These bacteria may harbor different genetic determinants which may be transferred to other bacteria including pathogens. Correspondingly, prevention strategies are required to surpass the stretch of these bacteria in the environment and community and to prevent the transfer of the antibiotic resistance to other enteric pathogenic bacteria.

Conflict of interest

This research didn't get any spesific funds. It was a self financed project. Authors declare no conflict of interest.

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