Bacteriology & Parasitology

Parvez et al., J Bacteriol Parasitol 2016, 7:2
http://dx.doi.org/10.4172/2155-9597.1000271

Short Communication Open Access

Prevalence of Inhibitor Resistant Beta Lactamase Producing *E. coli* in Human and Poultry Origin of Bangladesh

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Received date: March 29, 2016; **Accepted date:** April 21, 2016; **Published date:** April 29, 2016

Abstract

Human clinical specimens (n=48) and poultry fecal specimens (n=40) were collected from a sub district (Savar) of Bangladesh. Total 25 *E. coli* were isolated from these specimens. These *E. coli* were tested for their antibiotic sensitivity against commonly used antibiotics. In addition, to detect the ESBL (extended spectrum beta lactamases) producers, double disk synergy (DDS) test and PCR method were used. Twenty three *E. coli* were multidrug resistant, i.e. resistant against at least three different groups of antibiotics. DDS method showed all *E. coli* from poultry specimens and 78% of *E. coli* from clinical specimens were resistant to amoxicillin-clavulanic acid combination. Thus they are phenotypically confirmed as inhibitor resistant beta lactamase producers. PCR result showed only blaTEMgene in *E. coli* from poultry specimens. Thus inhibitor resistant type β-lactamase was found to be dominant in *E. coli* from both types of specimens.

Keywords: MDR; *E. coli*; Inhibitor resistant β-lactamase

Introduction

In Bangladesh high prevalence of multidrug resistant (MDR) and extended spectrum beta-lactamase (ESBL) producing bacteria in clinical specimens, hospital waste water, and drinking water samples have been reported [1-3]. The control of infection of human with Gram negative pathogenic bacteria (e.g. Enterobacteriaceae) can be seriously affected by high prevalence of antibiotic resistance and ESBL producing nature [4]. In developing countries like Bangladesh, the availability and indiscriminate use of antibiotics in clinical and non-clinical purposes increases the chance of antimicrobial exposure of the microorganisms and thus the inevitable increase in antibiotic resistance [5]. Among the various sectors in Bangladesh, poultry industry is one of the sectors where antibiotic misuse is common and thereby, have generated selection pressure towards antimicrobial resistance [6]. In fact, current data of Bangladesh have indicated presence of resistant and ESBL producing bacteria among poultry and household birds [7,8]. Recently in different countries poultry has been identified as a potential reservoir of resistant bacteria and transfer of resistance from non-pathogenic bacteria to pathogens and vice versa can happen [9,10]. In Bangladesh there is limited data on this perspective.

The aim of this study was to isolate and identify MDR and ESBL producing *E. coli* from fecal specimens of poultry and from clinical specimens of human to provide some baseline data in this perspective.

Materials & Methods

Sample collection

Clinical specimens were collected from a local clinic at Savar, Dhaka. Poultry fecal samples were collected from four poultry farms of Savar, Dhaka. Microbiological work was carried out at Department of Microbiology, Jahangirnagar University, Dhaka. Molecular work was carried out at Environmental laboratory, icddr, b, Dhaka, Bangladesh.

We have collected 48 human clinical specimens (urine) from a local clinic and 40 fresh chicken droppings from four poultry farms (ten samples from each). The timeline for specimen collection was from April-2012 to September-2012. Organisms were isolated and identified through a series of biochemical tests according to standard procedures [11].

Antibiotic sensitivity test

Antibiotic susceptibility test was done with amoxicillin (10 µg), tetracycline (30 µg), sulfamethoxazole-trimethoprim (23.75+1.25 µg), ciprofloxacin (5 µg), levofloxacin (5 µg) and nitrofurantoin (300 µg) disks (Oxoid) by disk diffusion method. ESBL screening was done by double disc synergy (DDS) method [12].

Molecular analysis

Molecular characterization of *ESBL* genes (bla_{CTX-M}, bla_{SHV}, bla_{TEM}, and bla_{OXA}) of 25 isolates were done through PCR. The PCR primers, annealing temperature and PCR condition used in this study were maintained according to references [13-16].

Results

14 *E. coli* and 11 *E. coli* were identified respectively from clinical and poultry specimens. Out of 25 isolates, 23 *E. coli* were multidrug resistant, i.e. resistant against at least three different groups of antibiotics. Highest level of resistance was against amoxicillin (Table 1).

DDS method showed that all *E. coli* from poultry origin and 78% *E. coli* from clinical specimens were resistant to amoxicillin clavulanic acid, therefore, inhibited the synergistic effect of clavulanate and cephalosporin against the ESBL and gave negative DDS results. No *E. coli* were true ESBL producers, but they showed the inhibitor resistant β-lactamase phenotype.

In the PCR, *E. coli* of poultry origin were found to carry only bla_{TEM} gene. *E. coli* from human origin carried three types of β-lactamase genes (bla_{TEM}, n=7; bla_{CTX-M}, n=2; and bla_{OXA}, n=1) except bla_{SHV}.

Antibiotic tested	% of resistance (Human origin)	% of resistance (Poultry origin)
Amoxicillin	100%	100%
Tetracycline	73.3%	100%
Sulfamethoxazole-trimethoprim	90%	92.3%
Nitrofurantoin	40%	30.8%
Ciprofloxacin	80%	84.6%
Levofloxacin	66.7%	77%

Table 1: *E. coli* from both human and poultry origin showed high level of resistance against commonly used antibiotics.

Discussion

In our study more than 80% of *E. coli* from chicken and human specimens were multidrug resistant. The antibiogram result showed complete resistance of all *E. coli* to amoxicillin and *E. coli* from chicken were also completely resistant to tetracycline. In previous studies carried out on *E. coli* from poultry environment in Bangladesh showed resistance to penicillin (i.e. amoxicillin and ampicillin) in a range of 28%-88% and to tetracycline 45.5% to 58% [7,8]. Though lowest level of resistance (30.8%) was found against nitrofurantoin for *E. coli* of chicken origin, but it is still a matter of concern that how these isolates become resistant to this broad spectrum of antibiotic which is mostly used for human treatment purpose. All of the chicken *E. coli* and 78% of clinical *E. coli* were resistant to amoxicillin/clavulanic acid, thus DDS result was negative for ESBL phenotype and positive for inhibitor resistance phenotype. The genotype bla_{TEM} was the most prevalent (78%) genotype in all types of *E. coli*. Both phenotype and genotype is indicative of the presence of inhibitor resistance TEM type β-lactamase enzyme, however, sequencing of these genes might be more conclusive. In our study, chicken droppings were collected from healthy chicken and thus *E. coli* from these specimens can be described as non-pathogenic. Still high level of antibiotic resistance was found among these isolates. Moreover, all the *E. coli* from chicken showed similar phenotype (inhibitor resistance) and genotype (bla_{TEM}). These non pathogenic but resistance gene containing *E. coli* can easily enter the environment and as many antibiotic resistance genes including bla_{TEM} are plasmid mediated, they can be easily transferred between and within bacterial species [6]. Thus nonpathogenic fecal origin *E. coli* have potential to transfer the resistant genes to pathogenic strains of *E. coli* and other compatible bacteria within the environment. The chicken *E. coli* can also cross-contaminate the meat and can directly enter the human body through food chain as previously reported [6,7]. The *E. coli* from clinical specimens were pathogenic as they were collected from infected patients' urine. The prevalence of inhibitor type beta lactamase phenotype (78%) in *E. coli* from clinical specimens was detected like the chicken derived *E. coli*. Presence of bla_{TEM} was also highest (50%, n=7) among *E. coli* from human origin. Though the genetic similarity between the resistant genes of chicken origin and human origin were not explored, still detection of same phenotype and β-lactamases genotype among the *E. coli* from both sources strongly suggest epidemiological linkage which is highly significant. Also SHV is a β-lactamase known to be frequent in clinical isolates [17], however, none of our clinical isolates harboured $blka_{SHV}$. The reason behind high prevalence of inhibitor resistance β-lactamase enzyme is unclear. If not controlled, transmission of multidrug resistance and beta lactamase genes from poultry environment to the human community can be a major public health challenge for Bangladesh as found in other countries [6,7]. Infection with drug resistant bacteria leads to higher mortality and treatment cost and has serious public health implications [18]. Such prevalence of antibiotic resistance demands continuous surveillance to determine the causes, sources and transmission of resistance.

Impacts

- High prevalence of inhibitor resistant β-lactamase phenotype and genotype has been found in *E. coli* isolated from poultry fecal specimens and human clinical specimens.
- Isolated *E. coli* from both sources also showed multi drug resistance against commonly used antibiotics.
- Transfer of resistance genes from poultry environment to human community and vice versa can happen and demands surveillance.

Acknowledgement

Authors acknowledge the support of environmental laboratory, icddr, b for genotyping analysis.

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