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SHORT COMMUNICATION

Antibiotic resistance of *Escherichia coli* isolated from captive Bengal tigers at Safari parks in Bangladesh

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ABSTRACT

Objectives: The present study was carried out to assess the antibiotic resistance and to identify the resistance genes in *Escherichia coli* from captive Bengal tigers at two Safari parks in Bangladesh.

Materials and Methods: A number of 24 environmental fecal swab samples of Bengal tigers were collected from two different Safari parks in Bangladesh. For the isolation of *E. coli*, samples were submitted to a number of bacteriological screening and biochemical tests. The antibiotic susceptibility of *E. coli* isolates was determined by disk diffusion method.

Results: Results demonstrated that 18 environmental fecal samples were positive to *E. coli* in bacteriological screening and biochemical test. The overall prevalence of *E. coli* in Bengal tiger was 75% (*n* = 18/24). The antibiogram study unveiled that all the isolates were resistant to ampicillin. Sulfamethoxazole-trimethoprim, nalidixic acid, and tetracycline were 89% (*n* = 16/18) resistant. On the contrary, 100% (*n* = 18/18) of the isolates were sensitive to colistin sulfate. bla_{TEM} was detected in 78% (*n* = 14/18) ampicillin-resistant isolates, whereas *sul*2 was found in 31% (*n* = 5/16) of the sulfamethoxazole-trimethoprim-resistant isolates.

Conclusion: This study, first time in Bangladesh, highlights a significant proportion of environmental fecal samples from captive Bengal tigers at Safari parks harboring antibiotic resistant *E. coli*. Transmission of resistant *E. coli* from Bengal tigers to humans and the environment could pose a public health risk at Safari parks in Bangladesh.

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Introduction

Antimicrobial drugs have been used for three major goals: to treat people and animals during infections, prophylactic use in people and animals, and sub-therapeutically use in food animals as growth promoters [1]. Indiscriminate use of antibiotics poses a selective pressure and leads to antimicrobial resistance that can be shared among bacterial populations [2]. The antibiotic resistance has been documented as a worldwide health problem for many decades [3].

Escherichia coli is a normal commensal bacterium in human and animals gut with some specific strains causing intestinal and extra-intestinal infections including cystitis, gastroenteritis, peritonitis, septicemia, and meningitis [4]. *Escherichia coli* is considered as a sentinel to scrutinize the resistance of antimicrobial agents in fecal bacteria due to its availability in a wide host range [5]. Commensal bacteria play a vital role to form resistance genes for the reservoir, which may convey between bacterial strains, including conveyance to those organisms competent to cause disease in humans and animals [6]. Once the antimicrobial-resistant *E. coli* can be found in the environment, migratory birds, wild animals, and invertebrates may further contribute to the dispersal of antibiotic-resistant genes [7]. Captive populations of Bengal tiger (*Panthera tigris tigris*) that are in close interaction with humans at Safari parks, being possible to transfer the resistant bacteria between humans and animals.

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Among five tiger subspecies, the Bengal tiger (*P. tigris tigris*) is the largest one. The Sundarbans (about 6,000 km^2) of Bangladesh and India are merely the mangrove forests settled by a generous tiger's population [8]. Safari parks were established to rescue and protect the world's endangered species like the Bengal tiger. Up until now, no information are available on the antibiotic resistance associated with resistance genes in *E. coli* from the Bengal tigers in Bangladesh. Hence, the current study was introduced to determine the antibiotic resistance and to identify two antibiotic resistance genes in *E. coli* from environmental fecal samples of Bengal tiger at Safari parks in Bangladesh.

Materials and Methods

Ethical statement

Ethical approval was not necessary for this study. Since fecal samples were collected from the environment without harming or giving stress to the animals.

Samples collection

Swab samples were collected aseptically from environmental fresh feces of Bengal tiger from Safari parks in Bangladesh, namely, Bangabandhu Sheikh Mujib Safari Park, Gazipur (number of tigers = 12, representative samples = 17) and Bangabandhu Sheikh Mujib Safari Park, Cox's Bazar (number of tigers = 4, representative samples = 7) during the period from January to March 2016. Each sample was placed into a sterile screw-capped falcon tube containing buffered peptone water (BPW). The samples were kept into an icebox and carried as early as possible to the Poultry Research and Training Centre (PRTC), Chattogram Veterinary and Animal Sciences University (CVASU).

Isolation of E. coli

The BPW (Oxoid, UK) containing sample was incubated for enrichment overnight at 37°C. One loop full of enriched broth from BPW was streaked onto MacConkey agar (Oxoid, UK), incubated for 18–24 h at 37°C. Single isolated colony from MacConkey agar was subjected onto Eosin Methylene Blue (EMB) agar (Merck, Mumbai), incubated at 37°C for 24 h. Among biochemical tests, indole production test, Methyl Red (MR) test, and Voges-Proskauer (VP) test were performed to confirm *E. coli*. The bacteria were preserved with 15% glycerol at −80°C until use.

Antibiotic susceptibility testing

Disk diffusion technique was performed to detect the antibiotic susceptibility of *E. coli* isolates on Muller-Hinton agar (Oxoid, UK) plate according to the guidelines and recommendations of CLSI [9]. The following 10 antibiotics were tested: ampicillin (10 µg), ceftriaxone (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), sulfomathoxazole-trimethoprim (25 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), colistin sulfate (10 µg), and erythromycin (15 µg) (HiMedia, India). The susceptibility results were interpreted according to the CLSI guidelines [9].

Amplification of antibiotic resistance genes

Boiling method was used for DNA extraction [10]. Each isolate was suspended with 150 µl of distilled water in an autoclaved Eppendorf tube, boiled for 10 min at 100°C. After boiling, the sample was placed onto the ice for 10 min for immediate heat shock. Then, the sample was re-centrifuged at 10,000 rpm for 5 min. The collected supernatant was used as DNA template. The primers with the target amplicon sizes used in this study are represented in Table 1.

To amplify resistant genes in *E. coli* isolates, a 25 µl total volume of PCR reaction mixture was prepared with 12.5 µl DreamTaq PCR Master Mix (Thermo Scientific, USA), 0.5 µl of each primer, 1 µl template DNA, and 10.5-µl deionized water. For amplification of bla_{TEM} gene, conditions were as follows: 30 cycles with the initial temperature at 94°C for 4 min, denaturation for 1 min at 94°C, annealing for 1 min at 60°C, elongation for 1 min at 72°C, and final extension at 72°C for 5 min. For *sul*2 gene, the amplification was conducted for 30 cycles with the initial temperature at 94°C for 5 min, followed by 94°C for 1 min, 59°C for 1 min, 72°C for 1 min, and 72°C for 7 min. PCR was carried out by a Thermocycler (2720 Thermal cycler, Applied Biosystems, USA). Products of PCR were electrophoresed using 1.5% agarose gel, stained with ethidium bromide (Sigma-Aldrich, USA), and finally, visualized by UV transilluminator (BDA Digital, Biometra GmbH, Germany).

Data analysis

All data were recorded into a spreadsheet of Microsoft Office Excel 2007 and shifted to QuickCalcsGraphpad software for data summary and descriptive statistics.

Results

Prevalence and cultural characteristics of E. coli

A number of 24 fecal swab samples were cultured. The overall prevalence was 75% (n = 18/24) (Table 2). *Escherichia*

Table 1. Primers used to identify antibiotic-resistant genes, bla_{TEM} and *sul*2.

Target genes	Primers sequence (5'-3')	Amplicon size	References
bla_{TEM}	F: TACGATACGGGAGGGCTTAC R: TTCCTGTTTTTGCTCACCCA	716-bp	Belaaouajet al. [11]
*sul*2	F: GAAGCGCAGCCGCAATTCAT R: TGTGCGGATGAAGTCAGCTC	435-bp	Change et al. [12]

coli on MacConkey agar produced bright pink colonies and typical green colonies with the metallic sheen on EMB agar. *Escherichia coli* were positive to MR and indole production whereas negative to VP test.

Antibiotic susceptibility test

All of the tested isolates were found resistant to ampicillin. 89% (n = 16/18) isolates were shown resistant to sulfamethoxazole-trimethoprim, nalidixic acid, and tetracycline. Erythromycin and chloramphenicol were resistant to 78% (n = 14/18) and 61% (n = 11/18), respectively. All the isolates were 100% (n = 18/18) sensitive to colistin sulfate followed by ceftriaxone (78%, n = 14/18), ciprofloxacin (39%, n = 7/18), and gentamycin (28%, n = 5/18). None of the isolates were sensitive to sulfamethoxazole-trimethoprim, nalidixic acid, ampicillin, and tetracycline. Antibiotic susceptibility of 18 isolates to different antibiotics is illustrated in Figure 1.

Antibiotic resistance genes

We have detected two of our targeting antibiotic resistance genes, namely, bla_{TEM} (β-lactamase resistance genes) and *sul*2 (sulfur drug resistance gene). Out of 18 ampicillin-resistant isolates, 14 gave positive amplicons for the bla_{TEM} gene (Fig. 2). Five of the 16 sulfamethoxazole-trimethoprim-resistant isolates contained the *sul*2 gene (Fig. 3).

Discussion

Antibiotic resistance is a serious consequence in the environment due to the overuse and misuse of antibiotics. Environmental bacteria have been rendered as a reservoir of antibiotic-resistant genes at distinct ecological niches and act as a significant root of resistant genes in other clinical microorganisms [13,14]. The overall prevalence in *E. coli* isolated from environment fecal samples of Bengal tigers was 75%. This is the maiden report describing the prevalence of *E. coli* in Bengal tigers at Safari parks in Bangladesh. Antibiotic susceptibility results of *E. coli* isolates to 10 different antibiotic agents were disclosed in this study. 100% isolates were resistant to ampicillin. 89% isolates were resistant to tetracycline, nalidixic acid, and sulfamethoxazole-trimethoprim, individually. High resistance also ascertained against erythromycin (78%)

Table 2. Prevalence of *E. coli* in two different Safari parks.

Name of Safari park	No. of sample	No. of positive	Prevalence (%)	95% CI
Bangabandhu Sheikh Mujib Safari park, Gazipur	17	14	82.35	58.16–94.62
Bangabandhu Sheikh Mujib Safari park, Cox's Bazar	7	4	57.14	25–84.25
Total	24	18	75	54.79–88.31

CI: Confidence Interval.

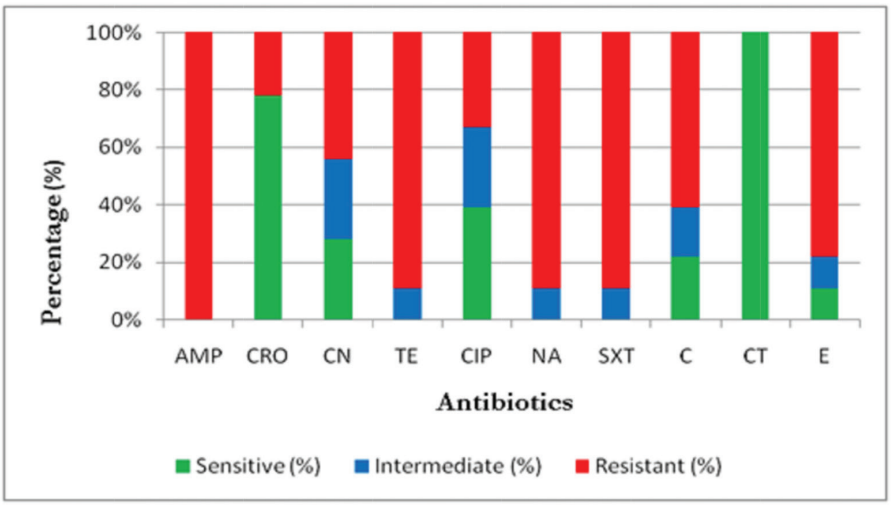

Figure 1. Antibiogram profile of *E. coli* isolated from Bengal tigers. AMP = Ampicillin, CRO = Ceftriaxone, CN = Gentamycin, TE = Tetracycline, CIP = Ciprofloxacin, NA = Nalidixic acid, SXT = Sulfamethoxazole-trimethoprim, C = Chloramphenicol, CT = Colistin sulfate, and E = Erythromycin.

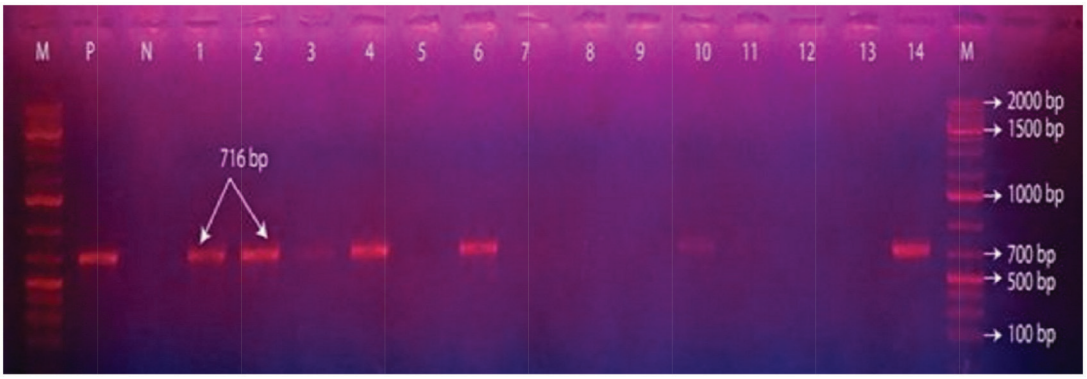

Figure 2. Amplification of *bla*$_{TEM}$ gene (716-bp) from the *E. coli* isolated from Bengal tigers. (Lane M: 100 bp ladder (Invitrogen); lane P: positive control; lane N: negative control; and lane 1, 2, 3, 4, 6, 10, 14: positive for *bla*$_{TEM}$ gene).

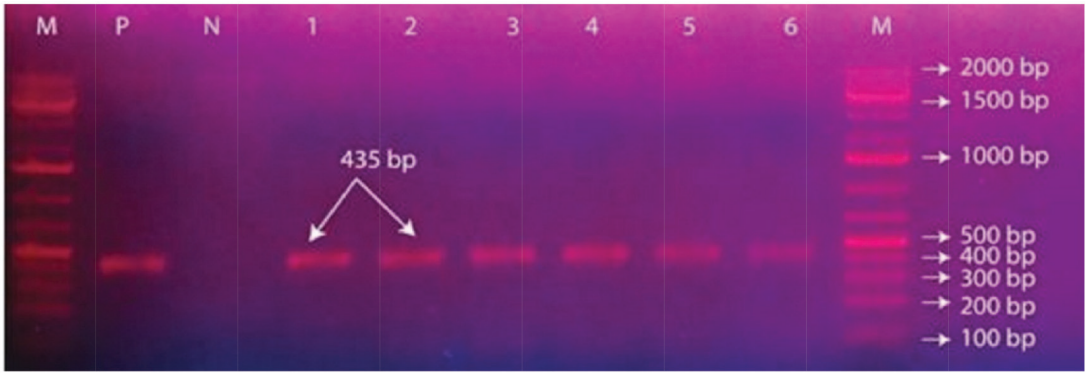

Figure 3. Amplification of *sul*2 gene (435-bp) from the *E. coli* isolated from Bengal tigers (Lane M: 100 bp ladder; lane P: positive control; lane N: negative control; and lane 1–6: positive for *Sul*2 gene).

and chloramphenicol (61%). The discovery of this study was in the line to the data obtained from the Heilongjiang Amur Tiger Park area in China [15]. Although tigers were administered antibiotics very rarely at Safari parks, results brought out high resistance scenery to different tested antibiotics. This may be due to the deforestation and spillage of organisms from human dwellings and wildlife in both directions. Moreover, the studied area is in the main bird migration route in Bangladesh and nearby lakes host thousands of wild birds. These birds picked up foods from different environments and human surroundings that are heavily polluted by resistant bacteria in Bangladesh [16]. Wild birds are considered as a reservoir and dissemination of resistant bacteria in a wildlife environment that could be an important reason how tigers acquired resistance of human-associated antibiotics.

However, all the isolates showed 100% sensitivity to colistin sulfate followed by ceftriaxone (78%) and ciprofloxacin (39%), according to Xue et al. [17], who reported 52% susceptibility against ciprofloxacin. Although colistin sulfate was susceptible to all isolates in our study, the last resort drug is being used extensively in agriculture and veterinary medicine [18].

In our present study, we have detected the beta-lactamase gene, *bla*$_{TEM}$ (78%), and sulfur drug-resistant gene, *sul*2 (31%). To the best of author's knowledge based on the rigorous literature searches, there are no previous reports on antibiotic resistant associated with genes in the scientific literature in *E. coli* from the captive population of tigers at Safari parks in Bangladesh. However, Xue et al. [17] found 80% *bla*$_{TEM}$ gene in their study in China, which supported our result. Antibiotic-resistant genes are transferred horizontally through food chains. For the past few years, an intensive and large-scale chicken and cattle farming industry has developed in Bangladesh. Resembling to our study, antibiotic resistances were found in *E. coli* isolates in environmental and biological sources such as human urine, human feces, sheep, goat, cattle, broiler, pigeon, duck, soil, and drain sewage in Bangladesh [19–21]. Beef meat is supplied as a principle diet of tigers at Safari parks. We have speculated that antibiotic resistance might have transferred through *E. coli* contaminated beef. The spreading of

resistance genes may be a great threat to the effectualness of antibiotic therapeutic agents at Safari parks in Bangladesh.

Conclusion

The study, for the very first time in Bangladesh, disclosed the high frequency of antibiotic resistant and presence of bla_{TEM} and *sul2* genes in fecal samples of Bengal tigers at Safari parks in Bangladesh. Thus, potential efforts should be taken for the detection of antibiotic-resistant genes in *E. coli* from captive as well as domestic animals to establish effective antimicrobial surveillance in Bangladesh.

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Conflict of interest

None.

Authors' contribution

SKG and AA contrived the project. MSS, SKG, and HSMZN collected samples from Safari park. SKG and MSS were involved in laboratory works. MSS and ZBB performed data analysis and manuscript writing. MSS, AA, and AS critically reviewed the article. All the authors read and approved for publication.

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