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Research Article

Antibiogram of *Escherichia coli* Isolated from Stool Samples of Non-human Primates (*Macaca mulatta*)

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Abstract

Antibiotic resistance is one of the alarming issues concerning public health worldwide. Although wild animal populations such as non-human primates (NHPs) are not directly exposed to antibiotics, antibiotic resistance has been detected in them. Previous studies have revealed the possibility of bacterial exchange between NHPs and humans living nearby, including strains associated with intestinal pathology. This study aimed to detect antibiotic-resistant *E. coli* prevalent as a gut flora in NHPs which show genetic and physiological similarities to humans. For this study, fresh stool samples of *Macaca mulatta* (*M. mulatta*) were collected from different sites in Kathmandu valley. A total of 25 samples were collected and 21 distinct colonies of *E. coli* were isolated and identified. After antibiogram of thus obtained colonies, 5 (23.81%) of the isolates were found to be resistant to Cefotaxime, all of them resistant to Ceftazidime, 3 (14.29%) resistant to Cefazolin and Gentamicin, only one resistant to Chloramphenicol, and all the isolates were sensitive to Meropenem and Nalidixic acid which concluded only 3 (14.29%) isolates to be multi-drug resistant (MDR). A total of 15 (71.42%) isolates were also found to be Extended Spectrum β-lactamase (ESBL) producing, and 8 (38.1%) isolates were found to be resistant to Colistin. This study suggested the presence of antibiotic-resistant gut *E. coli* in NHPs distributed in various areas of Kathmandu valley which can be transferred to the environment and humans causing a public health threat in the future.

Keywords: Antibiotics; Colistin; ESBL; MDR; Non-Human Primates

Introduction

Escherichia coli are highly versatile bacteria capable of adapting to a wide range of ecological niches and colonizing a diverse range of hosts. *E. coli* colonizes the gastrointestinal tract of human and non-human primates as a commensal but some of its strains are capable of causing intestinal and extraintestinal infections [1]. Antibiotic resistance has evolved to be one of the basic medical conditions of the 21st century that compromises the compelling counteraction and treatment of a consistently expanding scope of diseases brought about by microbes, parasites, infections, and organisms that are no longer defenseless to the basic prescriptions used to treat them [2]. While *E. coli* is intrinsically susceptible to almost all clinically applicable antibiotics, this bacterial species has a high capacity for resistance gene accumulation, which occurs primarily through horizontal gene transfer.

Antibiotic resistance occurs when microorganisms are exposed to antibiotic drugs. Under the selective pressure of antibiotics, susceptible bacteria are killed or inhibited, while bacteria that are intrinsically resistant or that have acquired antibiotic-resistant traits acquire a greater chance to survive and multiply [2]. The existence of resistant bacteria, resistance genes, antibiotics, and/or other selective pressures, such as heavy metals, in presumed antibiotic-free environments have been attributed to the human-mediated dispersal of resistant bacteria, resistance genes,

antibiotics, and/or other selective pressures [3]. The resistance developed can be against multiple classes of drugs i.e. multiple drug resistance (MDR). Some of the *E. coli* strains may produce extended-spectrum beta-lactamases (ESBL) which act against the beta-lactam antibiotics which include third-generation cephalosporins [4]. Likewise, in the case of colistin which is a last resort drug of choice for bacterial diseases, resistance rates among *E. coli* isolates have remarkably increased due to its use in veterinary medicine on a global scale [5].

M. mulatta is a common inhabitant species found in Nepal that comes in frequent contact with humans and residential areas. If *E. coli* commonly present in these species are found to have antibiotic resistance against multiple classes of drugs it would suggest that antibiotic resistance is not limited only to hospital environments and humans but it has spread to the environment and other animals. The transmission of these resistant strains to the environment and further to humans may lead to infections that cannot be treated with regular antibiotics or may be completely untreatable causing a public health threat.

Methods

A laboratory-based descriptive cross-sectional study was conducted. The samples were collected from five different places within the Kathmandu Valley. The areas selected for sampling were Pashupatinath, Bhandarkhal, Swayambhu, Bajrabarahi, and Nilbarahi. A total of 25 fresh stool samples (5 from each place) of *M. mulatta* were collected and processed during the study. 2gm of stool was mixed in 10 ml of saline solution. The emulsified sample was inoculated in Eosin Methylene Blue (EMB) Agar and incubated at 45°C for 24 hours. The greenish metallic sheen colonies were subcultured on Nutrient agar (NA) and MacConkey agar (MA) and incubated at 45°C for 24 hours. Biochemical tests were performed for the identification of *E. coli.* 0.5 McFarland was prepared with the isolated colonies. The inoculum was inoculated in the Mueller-Hinton Agar (MHA) plate and the antimicrobial discs [6]; Cefotaxime (30 mcg), Ceftazidime (30 mcg), Cefazolin (20 mcg), Gentamicin (10 mcg), Meropenem (10 mcg), Nalidixic acid (30 mcg), Chloramphenicol (30 mcg) and Cefotaxime/Clavulanic (30/10 mcg) were placed on an agar plate and incubated at 37°C for 18 hours. The zone of inhibition was measured and the results were interpreted as per CLSI 2022 guidelines [7]. The 0.5 McFarland inoculum was inoculated on MHA with 2µg/ml of colistin sulfate and the plates were incubated at 37ºC for 18 hours.

Result

Out of 25 samples, growth was observed in 24 (96%) samples. Among 24 samples with growth, 26 isolated typical colonies were obtained among which 21 (80.77%) isolates were identified as *E. coli*. All of the isolates were found to be resistant to Ceftazidime (30 mcg) and none of them were resistant to Meropenem(10 mcg) and Nalidixic acid (30 mcg) (Figure 1). 15 (71.42%) isolates were found to be ESBL producing (Figure 2) and 8 (38.1%) were found to be resistant to colistin (Figure 3); whereas 3 (14.29%) isolates were multi-drug resistant among all (Figure 4).

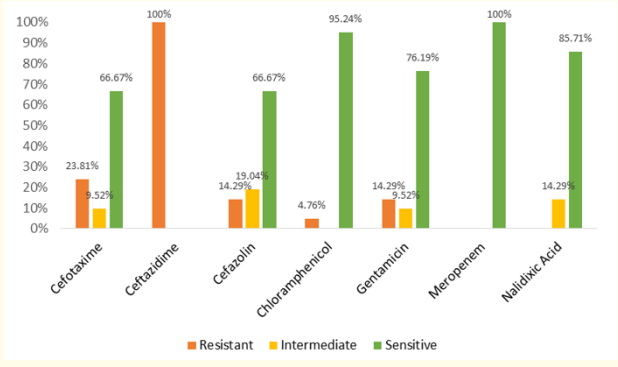


Figure 1: Antibiotic Susceptibility pattern of isolated *E. coli.*

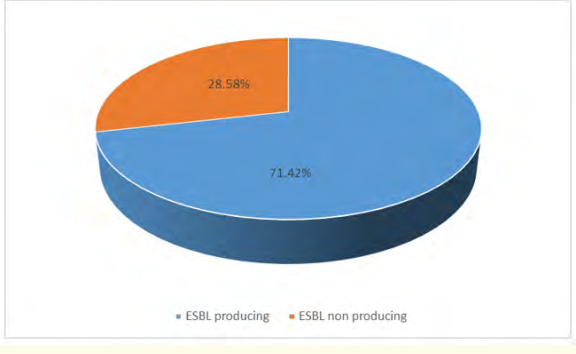


Figure 2: ESBL profile of Isolated *E. coli.*

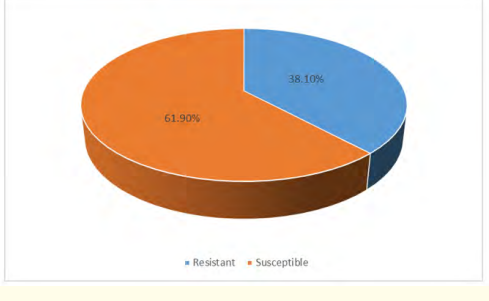


Figure 3: Colistin resistance profile of isolated *E. coli.*

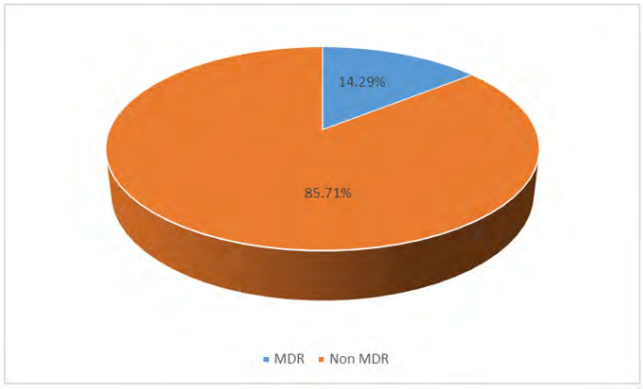


Figure 4: MDR profile of isolated *E. coli*.

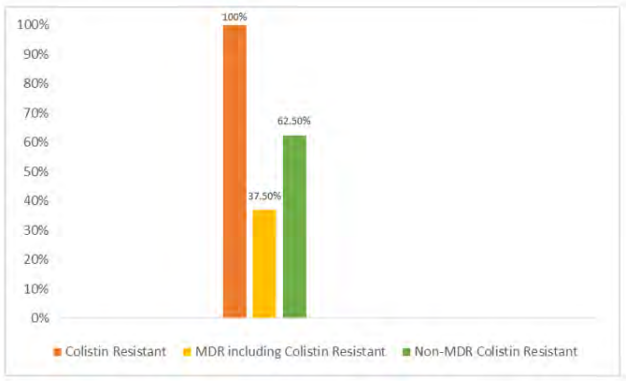
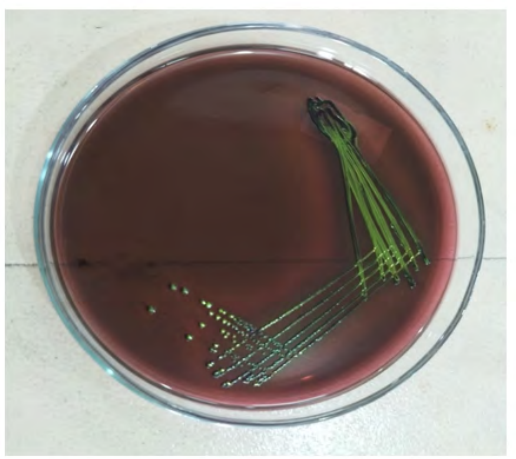
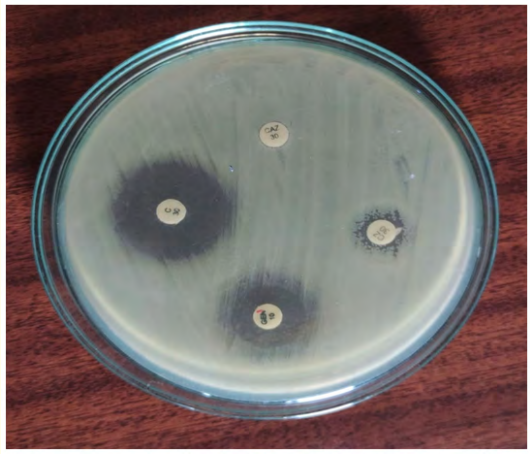


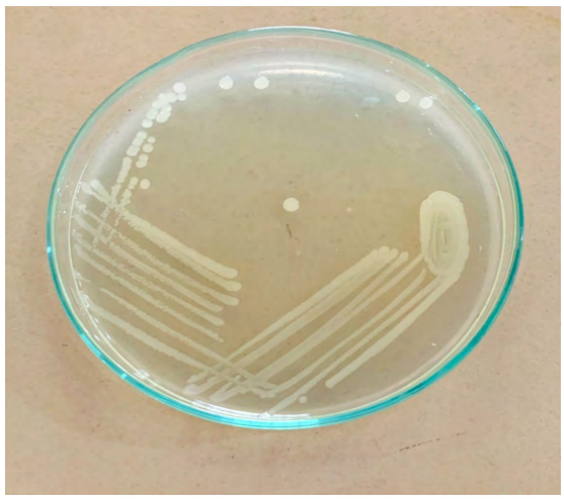
Figure 5: Chart representing MDR isolates inclusive of Colistin Resistant.



Photograph 1: Greenish Metallic *E. coli* Colonies on EMB Agar.



Photograph 2: AST by Kirby-Bauer Disc Diffusion Method.



Photograph 3: *E. coli* colonies on MHA with 2 µg/ml of Colistin.

Discussion

In the study carried out by Glover (2014), around 60% gut *E. coli* isolates of NHPs were resistant to Ceftazidime and 46.67% to Cefotaxime (30 mcg) which in case of our study was 100% and 23.81% respectively and for Cefazolin (20 mcg) to which 14.29% were resistant. Foster-Nyarko (2020) detected around 40% resistance against β-lactams in *E. coli* isolates from NHP in Gambia [1]. β-lactam antibiotics are commonly in use against infections of *E. coli* and the resistance can be generated by the production

of β-lactamase enzymes by the bacteria. In *E. coli*, β-lactamase mediated resistance is dominated mainly by the acquisition and dissemination of genomic elements through horizontal gene transfer via conjugation, transformation and transduction [8,9].

In our study, the majority of the isolates (71.42%), were found to be ESBL producers, which may have been responsible for the high resistance against third-generation Cephalosporin, Ceftazidime, in contrast, to the study conducted by Albrechtova., *et al.* (2014) in Tai National Park, which found zero ESBL producing strains in wildlife, including NHPs [10]. Rawat and Nair (2010) studied that ESBL encoding genes carry other antimicrobial agents showing resistance to aminoglycosides, tetracyclines, trimethoprim, sulphonamides, and chloramphenicol [11]. In accordance with their study, the one isolate resistant to Chloramphenicol in our study was ESBL producing and two among three isolates resistant to Gentamicin (Aminoglycoside) were ESBL producing. Different mechanisms have been proposed as being responsible for the acquisition of Ceftazidime resistance in *E. coli*, which is frequently associated with the presence of ESBLs or AmpC β-lactamase hyperproduction in conjunction with active efflux and/or decreased permeability [12-14].

Weiss (2018) detected that the decrease in proportion of resistant isolates from wild NHPs in rural Uganda was statistically significant. It was non-linear with increasing local antibiotic prices, with the most expensive antibiotics (Nalidixic acid and Ciprofloxacin) associated with very low resistance proportions (0.8% and 0.4% of isolates respectively) which is in accordance with our study with none of the isolates showing resistance against Nalidixic acid (30 mcg) [15]. Additionally, there were no *E. coli* isolates resistant to Meropenem (10 mcg), in contrast to Glover's study from 2014, where about 20% of the *E. coli* isolates were resistant [16].

Foster-Nyarko., *et al*. (2020) in his study discovered that 22 *E. coli* isolates encoded resistance genes to a single antibiotic agent, 22 isolates to two antibiotic classes, and only three isolates to three or more antibiotic classes i.e. only three of them showed MDR [1], which was similar to our study where MDR was seen in few isolates i.e. only three isolates and the most common MDR resistance profile included combined resistance to Ceftazidime, Gentamicin and Colistin in two isolates and Ceftazidime, Cefazolin, Chloramphenicol and Colistin for one isolate.

The antibiotic resistance demonstrated by the gut *E. coli* isolates in this project against third-generation Cephalosporins and last resort drug colistin is concerning because of the reliance of human antimicrobial treatment programs on these drugs. The treatment of infections associated with colistin resistant isolates can be through the combination of drugs such as Tigecycline [17]. Resistance to a specific antibiotic can be acquired through chromosomal DNA mutations or by mobile genetic elements. In the latter case, the exchange of genetic material between bacteria results in the spread of resistance [18,19].

Some studies imply that antimicrobial-resistant gut bacteria found in feces of free-roaming animals may be the result of direct or indirect contact with anthropogenic sources such as human foods as well as pollution in the environment from human wastes and/or wastes contaminated with antimicrobial residues [20]. In this study, we were unable to collect *E. coli* from humans who lived near the study primates. Comparisons of these isolates and sympatric human isolates may shed light on possible transmission routes between humans and primates in this context.

Conclusion

This study confirmed the presence of antibiotic-resistant bacteria in the gastrointestinal microbiota i.e. *E. coli* of free-roaming *M. mulatta* in Kathmandu valley. The results from the study determined the presence of MDR in *E. coli* isolated from the stool sample of the NHPs. The common drug against which all the isolates showed resistance was Ceftazidime whereas none of them showed resistance to Meropenem and Nalidixic acid. The presence of ESBL-producing *E. coli* and the Colistin-resistant *E. coli* were also detected. The chance of transfer of these strains is more due to frequent human contact with NHPs in places like Kathmandu valley which can put the health of the public at risk by causing infections caused by the bacteria to be hard to treat or untreatable.

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