ESCHERICHIA COLI–PRODUCING EXTENDED-SPECTRUM BETA-LACTAMASE CTX-M-15 IN A CAPTIVE SOUTH AMERICAN TAPIR (TAPIRUS TERRESTRIS)


Published By: American Association of Zoo Veterinarians
DOI: http://dx.doi.org/10.1638/1042-7260-44.1.173
URL: http://www.bioone.org/doi/full/10.1638/1042-7260-44.1.173

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne’s Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.
ESCHERICHIA COLI–PRODUCING EXTENDED-SPECTRUM BETA-LACTAMASE CTX-M-15 IN A CAPTIVE SOUTH AMERICAN TAPIR (TAPIRUS TERRESTRIS)


Abstract: Only a few reports exist on the occurrence of resistant bacteria in zoo animals. Therefore, an isolation of multiresistant Escherichia coli from the lungs of a captive South American tapir (Tapirus terrestris) lead to its characterization and further investigation of samples from animals inhabiting the same paddock and from the shared environment. The tapir suffered from an intermandibular abscess and pneumonia and was euthanatized after unsuccessful therapy, including administration of antibiotics. The authors performed selective isolation of extended-spectrum beta-lactamase (ESBL)–positive E. coli strains and identification of resistance genes using polymerase chain reaction. Seven multiresistant, ESBL-producing E. coli isolates were obtained, all belonging to the B2 phylogenetic group and showing identical profile on pulsed-field gel electrophoresis. These isolates carried several resistance genes, including the gene blaCTX-M-15. This case demonstrates the transmission of related epidemiologically important E. coli isolates whose potential transmission to other animals and zoo staff can be assumed.

Key words: Antibiotic resistance, Escherichia coli, extended-spectrum beta-lactamase, South American tapir, Tapirus terrestris.

BRIEF COMMUNICATION

Despite the growing importance of antibiotic resistance and attention recently given to this topic, only a few reports exist on the occurrence of resistant bacteria in zoo animals. In the present study, a multiresistant Escherichia coli isolate in a 5-yr-old male South American tapir (Tapirus terrestris) residing at the Brno Zoological Garden, Czech Republic, is described.

The animal suffered from diphtheroid-ulcerative stomatitis, swelling and prolapse of the tongue, and intermandibular abscess. After 3 wk of unsuccessful therapy, including administration of antibiotics (parenteral penicillin, gentamicin, enrofloxacin, and mouth rinsing with trimethoprim-sulfonamides), the animal was euthanatized because of its deteriorating condition. Fibrinopurulent tracheobronchitis and acute purulent pneumonia were found on postmortem examination. Bacteriologic culture from the lungs yielded a multiresistant E. coli isolate harboring the gene encoding production of the extended-spectrum beta-lactamase (ESBL) CTX-M-15. The tapir had come from the Gdansk Zoo (Poland) 4 yr earlier and was housed together with a female obtained from the Riga Zoo (Latvia) and their young, born in Brno. The three tapirs shared the paddock with three greater rheas (Rhea americana), a pair of capybaras (Hydrochoerus hydrochaeris), a pair of coscoroba swans (Coscoroba coscoroba), and a pair of upland geese (Chloephaga picta). In addition to samples from the dead tapir, the authors examined a total of 18 additional samples from other animals and their environment (mouth swabs from the female tapir and her young and pooled samples of feces from both tapirs, capybaras, rheas, and swans, respectively). Eight swabs from the housing of tapirs and capybaras, and four swabs from the shared paddock were also obtained. Selective isolation of ESBL-positive E. coli strains and their subsequent characterization using polymerase chain reaction for identifying selected resistance genes and phylogroup were performed as described previously. The XbaI restriction profiles of chromosomal DNA extracted within agarose gel plugs were analyzed by pulsed-field gel electrophoresis. Transferability
of antibiotic resistance genes was tested by plate-mating conjugation experiments\(^{10}\) and heat shock transformation of plasmid DNA to DH5\(\alpha\) competent cells. Resistance-encoding plasmids were analyzed by restriction fragment length polymorphism patterns obtained by Eco\(RV\) digestion and by replicon typing.\(^2\) The ESBL-producing isolates were serotyped,\(^{5,11}\) and virulence factors were determined by serologic and molecular methods,\(^{6,12}\) including heat-stable and heat-labile enterotoxins; verotoxin; adherence factor intimin (\(eaeA\)); adhesins F4, F5, F6, F18, or F41; and genes for selected virulence factors associated with extraintestinal pathogenic \(E.\) coli.

A total of seven ESBL-producing \(E.\) coli isolates were obtained: one isolate from the lungs of the dead tapir, one isolate from the feces of rheas, one isolate from the feces of swans, one isolate from the tapir housing environment, and three isolates from the shared paddock. All isolates belonged to the B2 phylogenetic group and showed identical pulse profile on pulsed-field gel electrophoresis (Fig. 1). They were resistant to ampicillin, cephalexin, ciprofloxacin, nalidixic acid, and tetracycline and carried the antibiotic resistance genes \(bla_{CTX-M-15}\), \(tetA\), and \(aac(\beta)Ib-cr\), as well as the integrase gene \(intI1\). Some isolates also demonstrated resistance to amoxicillin-clavulanate, gentamicin, and ceftazidime, an exception being the lung isolate, which, on the other hand, was resistant to sulfonamides and trimethoprim-sulfoximethoxazole. Serotyping was not successful, except for one isolate from the environment that belonged to the O8 serotype. No genes for the virulence factors tested except for \(fimC\) were detected. Conjugative transfer of plasmids carrying the genes \(bla_{CTX-M-15}\) into recipient cells of \(E.\) coli and \(Salmonella\) was not demonstrated. The lung \(E.\) coli isolate differed from the other isolates by the absence of the gene \(bla_{OXA}\) and by the presence of the resistance gene \(aadA1\) and the gene \(intI2\). The genes \(bla_{CTX-M-15}\) together with \(tetA\) and \(aac(\beta)Ib-cr\) on the incompatibility group F plasmid obtained from transformants of this isolate were detected. The plasmid size was 105 kb.

This current case demonstrates the epidemiologic spread of multiresistant \(E.\) coli strains carrying the gene \(bla_{CTX-M-15}\). Although the above-characterized isolate was probably nonpathogenic and has not been confirmed as the primary cause of the tapir’s disease, potential transmission to other animals and zoo staff (animal keepers, veterinarians) could occur, as suggested by the

---

**Figure 1.** Pulsed-field profiles of the \(E.\) coli isolates obtained from the lungs of the tapir, other animals, and the environment. The gel plugs were embedded in 1% SEAKEM gold agarose and run on Biorad a pulsed-field gel electrophoresis apparatus under the following conditions: initial time, 2.2 sec; final time, 54.2 sec; start ratio, 1; voltage, 200 V (6 V/cm); run time, 19 hr. \(Salmonella\) Braenderup H9812 was used as the molecular weight standard.
occurrence of related *E. coli* isolates in animals sharing the paddock with the tapir. A similar case was described on a large-scale pig farm in Denmark, where transmission of *E. coli* strains carrying IncN plasmids that mediate cephalosporin resistance was demonstrated between pigs and farm workers. The findings of this current case suggest that the spread of resistant enteric bacteria in a captive environment is enhanced by a high density of animals in a limited space, where contact with feces is more frequent.

**Acknowledgments:** The study was funded by grant MSM6215712402 from the Ministry of Education, Youth, and Sports of the Czech Republic, grant P502/10/P083 of the Czech Science Foundation, and grant 64/2010/FVHE of the Internal Grant Agency, University of Veterinary and Pharmaceutical Sciences Brno. This study was partly supported by the project “CEITEC—Central European Institute of Technology” (CZ.1.05/1.1.00/02.0068) from the European Regional Development Fund. The authors are grateful for the cooperation of Dr. Stanislav Mazanek, Ph.D., veterinarian at the Brno Zoological Garden, and for the bacteriologic examination of postmortem material by Dr. Ludmila Kohoutova.

**LITERATURE CITED**


Received for publication 19 August 2011