Advanced Journal of Microbiology Research Volume 2014
Available online at http://advancedresearchjournals.org/AJMR


*Full Length Research Paper*

# Reservoirs of antibiotic-resistant Enterobacteriaceae among animals sympatric to humans in Senegal: extended-spectrum beta-lactamases in bacteria in a black rat (*Rattus rattus*)

**Ivan Literak[1*], Monika Dolejska[1], Alois Cizek[2], Chiekh Ahmed Tidiane Djigo[3], Adam Konecny[4,5] and Petr Koubek[4]**

[1]Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.
[2]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.
[3]Administration of the Niokolo Koba National Park, Tambacounda, Senegal.
[4]Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic.
[5]Centre de Biologie et de Gestion des Populations, INRA-IRD, Montferrier sur Lez, France.

Accepted 2 October, 2013

**In Senegal, rectal swabs from cattle, wild rodents and chiropterans were cultured for Enterobacteriaceae. Isolates were tested for susceptibility to antimicrobial agents. Two cefotaxime-resistant isolates were found in a black rat (*Rattus rattus*): a multiresistant *Escherichia coli* with $bla_{CTX-M-15}$ gene and *Enterobacter cloacae* resistant to ampicillin and cephalotin with $bla_{TEM-52b}$ gene. Sympatric rats in Senegal may represent an important reservoir for antibiotic-resistant. Enterobacteriaceae including extended-spectrum beta-lactamases producing isolates.**

**Key words**: Antibiotics, resistance, *Escherichia*, *Enterobacter*, rat, Senegal.

## INTRODUCTION

Recently, bacteria isolated from feces and rectal swabs of wild and domestic animals in Europe and North America were examined for antimicrobial susceptibility. Close associations were found between occurrences of resistant bacteria in humans, domestic and sympatric wild animals (Skurnik et al., 2006). This may have been influenced by the use of antimicrobial agents. Data regarding occurrence of antibiotic-resistant bacteria in African domestic and wild mammals are limited. We examined cattle, wild rodents and chiropterans sympatric to humans in southeastern Senegal for the presence of antibiotic-resistant Enterobacteriaceae. Our results are presented and discussed in this paper.

*Corresponding author. E-mail: literaki@vfu.cz. Tel: +420541 562 630. Fax: +420 541 652 631.

## MATERIAL AND METHODS

In September 2007, we examined domestic cattle (n = 48; 31 cows, 4 haifers, and 13 calves) , wild rodents (n = 45; *Rattus rattus* 33, *Arvicola ansorgei* 4, *Myomys daltoni* 3, *Mastomys erythroleucos* 2, *Tatera guinea* 2, *Mus musculus* 1) and chiropterans (n = 24; *Epomorphorus gambianus* 19, *Micropteropus pusilus* 3, *Hiposideros gigas* 2) sympatric to humans, from two locations in southeastern Senegal for the presence of antibiotic-resistant Enterobacteriaceae. Wild rodents and chiropterans were caught using live traps and mist nets, respectively, in places inhabited by humans. Rectal swabs obtained from all the sampled animals were stored in Amies transport medium before laboratory testing.

Individual rectal swabs were placed overnight in Buffered Peptone Water (BPW) (Oxoid, UK) at 37°C, and then cultured for *E. coli* on Chromogenic medium for *E. coli* and coliform bacteria (Oxoid). One suspect colony of each plate was identified using API10S test kit (bioMérieux, France) and tested for susceptibility to antimicrobial agents in accordance with CLSI (The Clinical and Laboratory Standards Institute). Antibiotic susceptibility was tested by disk diffusion method on Mueller-Hinton agar (Oxoid) using the

following antimicrobials and concentrations: amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), cephalothin (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), sulphamethoxazole-trimethoprim (25 µg), sulphonamides (300 µg), and tetracycline (30 µg) (Oxoid).In *E. coli* isolates found to be resistant to one or more of the antibiotics listed above, polymerase chain reaction (PCR) was used to detect specific antibiotic-resistance genes, integrase genes *int*1 and *int2*, and gene cassettes within class 1 and 2 integrons (Dolejska et al. 2007, for the list of primers see Literak et al., in press).

For isolation of extended-spectrum beta- lactamase-producing and quinolone-resistant Enterobacteriaceae, the samples were enriched overnight at 37°C in MacConkey broth (Oxoid) and after that subcultured on MacConkey agar containing cefotaxime (2 mg $L^{-1}$) and MacConkey agar containing nalidixic acid (20 mg $L^{-1}$) to detect Enterobacteriaceae resistant to cefotaxime and nalidixic acid, respectively. The cefotaxime-resistant colonies were examined using the double-disk synergy test for the production of extended-spectrum beta-lactamase (ESBL) (CLSI 2002) and identified by the API 10S test kit (bioMérieux). The genes responsible for the ESBL phenotype (*bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$) were identified by PCR and the products were further analysed using sequencing (ABI 310 Genetic Analyser, Applied Biosystems). The primers 1537 and 1580 were used for sequencing of *bla*$_{CTX-M-1\ group}$ and the primers 686 and 757 were used for sequence analysis of *bla*$_{TEM}$ (Literak et al., in press). Transferability of *bla* genes was tested by conjugation (Literak et al., in press). Plate-mating experiments were done using plasmid-free, rifampicin and nalidixic acid resistant *E. coli* MT102RN and *Salmonella* Typhimurium SL5325 as recipients (Caroff et al., 1999; Olesen et al., 2004).

## RESULTS AND DISCUSSION

A total of 48 isolates of *E. coli* were obtained from 48 samples (one sample - one isolate) of cattle in Dar Salam (13°15´ N, 13°12´ W). Three isolates were resistant to tetracycline encoded by the *tetA* gene. Using a selective cultivation, one more *E. coli* isolate resistant to nalidixic acid but susceptible to ciprofloxacin was obtained. We do not consider the cattle in that area of Senegal to comprise an important reservoir of resistant *E. coli* nor as a risk factor for transmitting resistant *E. coli* to people living in close contact with their cattle. It should be noted that an *E. coli* isolate resistant to tetracycline originating from cattle has been documented in Kenya, which is also in tropical Africa (Kikuvi et al., 2007).

A total of 37 and 24 *E. coli* isolates from 37 wild rodents and 24 chiropterans respectively, in Dar Salam were obtained by cultivation on Chromogenic medium. Two (5%) isolates from wild rodents, both from black rats (*Rattus rattus*), were resistant: one resistant to tetracycline carrying the *tetA* gene and one multiresistant to sulphonamides, sulphametoxazole-trimethoprim and tetracycline with the *sul2* and *tetA* genes. Only one (4%) isolate from chiropterans (*Micropteropus pusillus* ) was resistant to tetracycline due to presence of the *tetA* gene. No antibiotic-resistant isolates were obtained using selective cultivation on media with cefotaxime or nalidixic acid. The prevalence of resistant isolates in both wild rodents and chiropterans in Dar Salam was low, and, as in cattle, the most frequent resistance was to tetracycline. The information on antimicrobial usage in people as well as in domestic animals in Dar Salam is limited, and there was probably only small pressure for selection of resistant *E. coli* in their populations and consequently for the spreading of resistant isolates or genetic determinants of resistance to sympatric populations of wild animals. A study in Indonesia showed that the use of antimicrobials in people and domestic animals and fecal contamination of the environment was associated with the isolation of resistant Enterobacteriaceae in sympatric wild rodents including black rats and chiropterans (Graves et al., 1988).

Additionally, eight wild rodents sympatric to human buildings were examined in Tambacounda (13°45´ N, 13°40´ W). Using cultivation on Chromogenic medium 8 *E. coli* isolates were obtained, however, resistance to antimicrobials tested was not found in any isolate. Using the selective method of cultivation on medium with cefotaxime or nalidixic acid, two *E. coli* isolates resistant to nalidixic acid and sensitive to ciprofloxacin were found in two black rats. Moreover two isolates of cefotaxime-resistant Enterobacteriaceae were found in another black rat. Found were an *E. coli* isolate resistant to ampicillin, streptomycin, sulphonamides, sulphametoxazole-trimethoprim and tetracycline with genes *bla*$_{CTX-M-15}$, *strA*, *sul2*, and *tetA*, as well as an *Enterobacter cloacae* isolate with the *bla*$_{TEM-52b}$ gene. The *bla* genes failed to transfer by conjugation into *E. coli* and *Salmonella*. Tambacounda is a town with poor hygienic conditions where wild rodents commonly inhabit buildings with humans and can be easily colonized with fecal bacterial strains excreted by humans or resistance determinants can be spread by horizontal gene transfer.

The dissemination of ESBLs is a problem of global magnitude, with rates of production being particularly high in some enterobacterial species, especially *Klebsiella pneumoniae* and *E. coli* (Rossolini et al., 2008). Various types of ESBLs (TEM, SHV, CTX- M) has been documented recently in human clinical isolates of *Enterobacteriacea* in different regions of Africa (Kariuki et al., 2001, Usha et al., 2008, Ehlers et al., 2009). The CTX-M- 15 beta-lactamase has been detected in hospital isolates of *Salmonella enterica* in Senegal (Weill et al., 2004), *Klebsiella pneumoniae* isolates connected with community-acquired urinary tract infections in Nigeria (Soge et al., 2006). CTX-M-15 is also wide spread in hospital isolates of *Klebsiella pneumoniae*, *E. coli* and *Enterobacter cloacae* in Algeria (Touati et al., 2006, 2007, Iabadene et al., 2008, Massai et al., 2008) and Tunisia (Ktari et al., 2006, Abbassi et al., 2008). Global dissemination of CTX -M-15 and importance of clonal spreading has recently been documented by Clermont et al. (2008). These authors reported a clone of CTX-M-15-producing *E. coli* spreading through Europe, Tunisia and Central African Republic.

Recently, two children with no known antibiotic exposure living in a remote Senegalese village, were found to be fecal carriers of a multiresistant *E. coli* clone that pro-

duced CTX-M-15 beta-lactamase (Ruppe et al., 2009). These isolates were able to transfer resistance to cephalosporins since the presence of $bla_{CTX\text{-}M\text{-}15}$ was confirmed by PCR in the transconjugants. This highlights the current massive spread of extended-spectrum beta-lactamases, especially CTX-M-15, even in isolated communities.

TEM-52 has been seen in non-typhoid *Salmonella* of human origin and *Salmonella* seems to be the preferred reservoir for this ESBL type (Yates et al., 2004; Hasman et al., 2005). Only few TEM- 52 producers are reported in animals. The gene $bla_{TEM\text{-}52}$ has been documented in *E. coli* from pets (Costa *et al.* 2004) and food-producing animals (Brinas *et al.* 2005), In the wild, the gene $bla_{TEM\text{-}52}$ has been detected in *E. coli* from wild birds and game in Portugal (Costa et al., 2008, Poeta et al., 2008) and wild boars in the Czech Republic (Literak et al., in press). To define the extent of the spread of ESBLs, the characterization of antibiotic-resistant bacteria needs to be done in each geographical area and different environments including wildlife, especially in areas where resources are limited and antibiotics are unregulated (Okeke et al., 1999).

We conclude that rats sympatric to humans in Senegal, similarly as rats in Kenya (Gakyua et al., 2001), represent a possibly important reservoir for antibiotic-resistant Enterobacteriaceae including ESBL producing strains.

## ACKNOWLEDGEMENTS

This study was funded by grants nos. MSM6215712402 (Ministry of Education, Youth and Sports of the Czech Republic) and IAA 6093403 (Grant Agency of the Academy of Sciences of the Czech Republic).

### REFERENCES

Abbassi MS, Torres C, Achour W, Vinue L, Saenz Y, Costa D, Bouchami O, BenHassen A (2008). Genetic characterisation of CTX-M-15-producing *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from stem cell transplant patients in Tunisia. Int. J. Antimicrob. Agents. 32: 308–314.

Brinas L, Moreno MA, Teshager T (2005). Monitoring and characterization of extended spectrum beta-lactamases in *Escherichia coli* strains from healthy and sick animals in Spain. Antimicrob. Agents Chemother. 49: 1262–1264.

Caroff N, Espaze E, Berard I, Richet H, Reynaud A (1999). Mutations in the ampC promoter of *Escherichia coli* isolates resistant to oxyiminocephalosporins without extended spectrum beta-lactamase production. FEMS Microbiol. Let. 173: 459–465.

Clermont O, Lavollay M, Vimont S, Deschamps C, Forestier C, Branger C, Denamur E, Arlet G (2008). The CTX-M-15-producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. J. Antimicrob. Chemother. 61: 1024–1028.

CLSI. Clinical and Laboratory Standards Institute Guidelines (2002) Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard M31-A2. National Committee for Clinical Laboratory Standards, Wayne, Pa.

Costa D, Poeta P, Brinas L, Saenz Y, Rodrigues J, Torres C (2004). Detection of CTX M-1 and TEM-52 beta-lactamases in *Escherichia coli* strains from healthy pets in Portugal. J. Antimicrob. Chemother. 54: 960–961.

Costa D, Poeta P, Saenz Y, Vinue L, Coelho AC, Matos M, Rojo-Bezares B, Rodrigues J, Torres C (2008). Mechanisms of antibiotic resistance in *Escherichia coli* isolates recovered from wild animals. Microb. Drug Resist. 14: 71–77.

Dolejska M, Cizek A, Literak I (2007). High prevalence of antimicrobial-resistant genes and integrons in *Escherichia coli* isolates from black-headed gulls in the Czech Republic. J. Appl. Microbiol. 103: 11–19.

Dolejska M, Bierosova B, Kohoutova L, Literak I, Cizek A (2009). Antibiotic-resistant *Salmonella* and *Escherichia coli* isolates with integrons and extended-spectrum beta- lactamases in surface water and sympatric black-headed gulls. J. Appl. Microbiol. 106: 1941–1950.

Ehlers MM, Veldsman C, Makgotlho EP, Dove MG, Hoosen AA, Kock MM (2009). Detection of $bla_{SHV}$, $bla_{TEM}$ and $bla_{CTX\text{-}M}$ antibiotic resistance genes in randomly selected bacterial pathogens from the Steve Biko Academic Hospital. FEMS Immunol. Med. Microbiol. 56: 191–196.

Gakuya FM, Kyule MN, Gathura PB, Kariuki S (2001). Antimicrobial susceptibility and plasmids from *Escherichia coli* isolated from rats. East Afr. Med. J. 78: 518–522.

Graves SR, Kennelly-Merrit SA, Tidemann CR, Rawlinson PA, Harvey KJ, Thornton IWB (1988). Antibiotic-resistance patterns of enteric bacteria of wild mammals on the Krakatau Island and West Java, Indonesia. Phil. Trans. R. Soc. Lond. B. 322: 339–353.

Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM (2005). Beta-lactamases among extended-spectrum beta-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. J. Antimicrob. Chemother. 56:115–121.

Iabadene H, Messai Y, Ammari H, Ramdani-Bouguessa N, Lounes S, Bakour R, Arlet G (2008). Dissemination of ESBL and Qnr determinants in *Enterobacter cloacae* in Algeria. J. Antimicrob. Chemother. 62: 133–136.

Kariuki S, Corkill JE, Revathi G, Musoke R, Hart CA (2001). Molecular characterization of a novel plasmid-encoded cefotaximase (CTX-M-12) found in clinical *Klebsiella pneumoniae* isolates from Kenya. Antimicrob. Agents Chemother. 45: 2141–2143.

Kikuvi GM, Schwarz S, Ombui NJ, Mitema ES, Kehrenberg C (2007). Streptomycin and chloramphenicol resistance genes in *Escherichia coli* isolates from cattle, pigs, and chicken in Kenya. Microb. Drug Resist. 13: 62–68.

Ktari S, Arlet G, Mnif B, Gautier V, Mahjoubi F, Ben Jmeaa M, Bouaziz M, Hammami A (2006). Emergence of multidrug-resistant *Klebsiella pneumoniae* isolates producing VIM-4 metallo-beta-lactamase, CTX-M-15 extended-spectrum beta-lactamase, and CMY-4 AmpC beta-lactamase in a Tunisian university hospital. Antimicrob. Agents Chemother. 50: 4198–4201.

Literak I, Dolejska M, Radimersky T, Klimes J, Friedman M, Aarestrup FM, Hasman H, Cizek A. Antimicrobial-resistant faecal *Escherichia coli* in wild mammals in central Europe: multiresistant *E. coli* producing extended-spectrum beta-lactamases in wild boars. J. Appl. Microbiol. In press.

Messai Y, Iabadene H, Benhassine T, Alouache S, Tazir M, Gautier V, Arlet G, Bakour R (2008). Prevalence and characterization of extended-spectrum beta-lactamases in *Klebsiella pneumoniae* in Algiers hospitals (Algeria). Pathol. Biol. 56: 319–325.

Okeke IN, Lamikanra A, Edelman R (1999). Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg. Infect. Dis. 5: 18–27.

Olesen I, Hasman H, Aarestrup FM (2004). Prevalence of beta-lactamases among ampicillin-resistant *Escherichia coli* and *Salmonella* isolated from food animals in Denmark. Microb. Drug Res. 10: 334–340.

Poeta P, Radhouani H, Igrejas G, Goncalves A, Carvalho C, Rodrigues J, Vinue, Somalo S, Torres C (2008). Seagulls of the Berlengas natural reserve of Portugal as carriers of fecal *Escherichia coli* harboring CTX-M and TEM extended-spectrum beta lactamases. Appl. Environ. Microbiol. 74: 7439–7441.

Rossolini GM, D'Andrea MM and Mugnaioli C (2008). The spread of CTX-M-typeextended-spectrum beta-lactamases. Clin. Microbiol.

Infect. 14(1): 33–41.
Ruppe E, Woerther PL, Diop A, Sene AM, Da Costa A, Arlet G, Andremont A, Rouveix B (2009). Carriage of CTX-M-15 – producing *Escherichia coli* among remote-living children in Senegal. Antimicrobial Agents Chemother. 53: 3135-3137.
Skurnik D, Ruimy R, Andremont A, Amorin C, Rouquet P, Picard B, Denamur E (2006). Effect of human vicinity on antimicrobial resistance and integrons in animal faecal *Escherichia coli*. J. Antimicrob. Chemother. 57: 1215–1219.
Soge O, Queenan AM, Ojo KK, Adeniyi BA, Roberts MC (2006). CTX-M-15 extended-spectrum beta-lactamase from Nigerian *Klebsiella pneumoniae*. J. Antimicrob. Chemother. 57: 24–30.
Touati A, Benallaoua S, Djoudi F, Madoux J, Brasme L, De Champs C (2006). Characterization of CTX-M-15-producing *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from hospital environments in Algeria. Microb. Drug Res. 13: 85–89.
Touati A, Benallaoua S, Djoudi F, Madoux J, Brasme L, De Champs C (2007). Characterization of CTX-M-15-producing *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from hospital environments in Algeria. Microb. Drug Res. 13: 85–89.
Usha G, Chunderika M, Prashini M, Willem SA, Yusuf ES (2008). Characterization of extended-spectrum beta-lactamases in *Salmonella spp.* at a tertiary hospital in Durban, South Africa. Diagn. Microbiol. Infect. Dis. 62: 86–91.
Weill FX, Perrier-Gros-Claude JD, Demartin M, Coignard S, Grimont PA (2004). Characterization of extended-spectrum-beta-lactamase (CTX-M-15)-producing strains of *Salmonella enterica* isolated in France and Senegal. FEMS Microbiol. Lett. 238: 353–358.
Yates CM, Brown DJ, Edwards GF, Amye SG (2004). Detection of TEM-52 in *Salmonella enterica* serovar Enteritidis isolated in Scotland. J. Antimicrob. Chemother. 53: 407–408.