Detection of genes mediating beta-lactamase production in isolates of enterobacteria recovered from wild pets in Saudi Arabia

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Abstract

Aim: To determine the genetic basis and types of beta-lactamase encountered among enterobacterial isolates of wild pets from the animal exhibit.

Materials and Methods: A total of 17 beta-lactamase-producing enterobacteria recovered from fecal samples of wild pet animals were analyzed for a selected beta-lactamase gene by polymerase chain reaction.

Results: Molecular analysis identified one or more beta-lactamase-encoding genes in 14 enterobacterial isolates as a single or gene combination. The most frequent extended-spectrum beta-lactamases types were TEM and CTX-M, and the most common AmpC enzymes were CMY-2 and DHA types.

Conclusions: The study is the first in Saudi Arabia, have established the presence of beta-lactamase-encoding genes in the fecal isolates of wild pets.

Keywords: animal exhibit, extended-spectrum beta-lactamases/AmpC beta-lactamase, fecal samples, polymerase chain reaction, Saudi Arabia.

Introduction

Antibiotic-resistant bacteria are extremely important to human health. The production of beta-lactamases is the major mechanism of bacterial resistance to beta-lactam antibiotics which are considered the most widely used class of antibiotics against both Gram-negative and Gram-positive bacteria. Resistance to this class of antimicrobial agents is therefore of immense clinical significance.

A major reason for resistance of Enterobacteriaceae to beta-lactam antibiotics is the production of extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases, capable of inactivating the effects of broad-spectrum cephalosporins and penicillins [1]. Exposure to ESBL/AmpC-producing microorganisms can occur through any means, but the hospital has always been thought to be the greatest risk [2]. The occurrence of ESBL/AmpC-producing microorganisms is on the rise globally, with prevalence varying from country to country and within a country from institution to institution [3]. The genes that encode for these enzymes may be plasmid-borne or chromosomally located.

Wild animals provide a biological mechanism for the spread of antibiotic resistance genes [4]. Recently, a number of studies describing the occurrence of ESBL-resistant Escherichia coli in wildlife [5-14].

Data from the Arabian Peninsula, including Saudi Arabia, suggested that extended-spectrum and AmpC beta-lactam-resistant bacteria constitute a major problem in nosocomial and community-acquired infections [15,16]. However, there is scarce information on the occurrence and genetic characteristics of beta-lactamase-producing bacteria in wild pet animals. Therefore, this study was carried to investigate the occurrence and distribution of beta-lactamase encoding genes within enterobacteria derived from wild pet animals in Saudi Arabia.

Materials and Methods

Ethical approval

The fecal samples were collected aseptically with adequate precautionary measures to minimize pain and/or discomfort to the animals and carried out in accordance with the Saudi animal welfare laws.

Bacterial strains

A total of 17 positive ESBL/AmpC enterobacterial isolates recovered from 75 fecal samples of wild animals at pet market, Taif, Western Saudi Arabia (5 rock hyrax, 4 Yemen Linnet, 3 common kestrel, 3 red foxes, 3 long-tailed finches, 2 caracal, 2 peacock, 1 rock dove, 1 hamadryas baboon, 1 orange-winged parrot, 1 Burmese python, 1 Hill Mynah, 1 African gray parrot, 1 common myna) were included. Wild animals are caught or bought for pet, shops, local breeder or traded (sometimes illegally). The enterobacterial isolates were 9 E. coli, and single isolates of Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, Proteus
vulgaris, Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii, and Citrobacter youngae. Isolates were identified and confirmed by commercially available biochemical test (API tests; bioMérieux). The ESBLs and AmpC beta-lactamase production were achieved by commercially available Etest (bioMérieux).

**Molecular investigation**

Rapid DNA preparation was performed by a boiling technique that includes heating at boiling of an overnight bacterial culture (200 μl) mixed with 800 μl of distilled water, followed by cooling, centrifugation and the supernatant was used as the DNA template for the polymerase chain reaction (PCR).

The presence of genes encoding TEM, SHV, OXA, CTX-M, CMY-2, and DHA type beta-lactamases was studied by multiplex PCR using universal primers and conditions previously reported [17,18]. The PCR was conducted in a Thermal Cycler PXE-0.5 (THERMO; Electron Corporation) and the resulting PCR products were subjected to electrophoretic separation in 1.5% agarose gel. Visualization of amplicons was completed by staining with ethidium bromide (Sigma-Aldrich) (1 μg/ml) under UV transluminator and photographed. DNA bands of each amplicon were compared with 100-bp DNA mass marker (Figure-1a-c). Primers sequence and PCR condition are presented in Table-1.

**Results**

**PCR detection of beta-lactamase encoding genes**

A total of 17 beta-lactamase positive enterobacterial strains recovered from the feces of wild pet animals were screened for beta-lactamase (bla) encoding genes. The PCR screening identified the presence of the beta-lactamase genes encoding TEM, CTX-M, CMY-2, and DHA in 14 of them (Figure-1a-c). None of the isolates were reacted positively for blaOXA and blashaV. No beta-lactamase genes were identified in the remaining three isolates.

Overall, variety of beta-lactamase genes were found within nine bacterial species isolated from various wild pets species. TEM enzyme was detected in nine isolates of beta-lactamase-producing, respectively, which included 4 isolates of E. coli and single isolate of E. aerogenes, P. mirabilis, C. youngae, and P. vulgaris (Table-2). The CTX-M enzyme was identified in five strains among of beta-lactamase-producing isolates, as a single isolate of E. coli, K. pneumonia, E. cloacae, K. oxytoca and C. freundii (Table-2). Both of CMY-2 and DHA, a plasmid-mediated AmpC beta-lactamases were detected in two different isolate of E. coli (Table-2).

**Distribution of bla genes**

The beta-lactamase-producing isolates were distributed into two categories, the first harbored only one type of beta-lactamase encoding gene, the second harbored two types (Table-2). Twelve (12/17) of the total beta-lactamase-producing enterobacteria were harboring only one beta-lactamase encoding gene, including five strains of E. coli and a single isolate of E. cloacae, K. oxytoca, C. youngae, P. vulgaris, C. freundii, P. mirabilis and E. aerogenes.

The blaTEM, a narrow-spectrum beta-lactamase was detected alone in 7 isolates; E. coli (3 isolates) and a single isolate of C. youngae, P. vulgaris, P. mirabilis and E. aerogenes. The blaCTX-M, an extended-spectrum beta-lactamase was detected alone in four isolates; single isolate of K. oxytoca from Yemen linnet feces, E. coli from common kestrel, E. cloacae from rock dove, and C. freundii from African gray parrot (Table-3). The plasmid-mediated beta-lactamases, blaCMY-2 and blaDHA were detected in two different E. coli isolates recovered from Arabian red fox and Hill Mynah, respectively.

A total of two (2/17) of the total beta-lactamase-producing isolates were harboring gene combinations of blaTEM and blaDHA in E. coli recovered from the feces of Hill Mynah and blaTEM and blaCTX-M in K. pneumonia delivered from the feces of baboon monkey.

**Discussion**

The resistance to beta-lactam and beta-lactamase inhibitors is of great clinical significance in several countries. Resistance to beta-lactam antibiotics is primarily mediated by beta-lactamases production. Many different beta-lactamases have been described, but TEM, SHV, OXA, CMY-2, and CTX-M beta-lactamases are currently regarded the most common among Enterobacteriaceae spp. [2].

Recently, many studies carried out in different countries describing the prevalence and characteristics of beta-lactamase gene harbored Enterobacteriaceae in wildlife free-living Canada geese in Georgia and North California [19], wild animals in Portugal [8,20], zoo animals in Japan [21], black-headed gulls in the
Since there seem to be geographical variations in the occurrence of different ESBLs, we describe prevalence and characteristics of ESBL/AmpC-genotypes within enterobacterial isolates from wild pet animals presenting at live animal market in Taif, Western Saudi Arabia. 

Prevalence of beta-lactamase genes

The beta-lactamase genes harboring enterobacterial isolates from wild pet animals were detected in...
whereas consistent with that previously reported [8,20,21], bla genes among enterobacteria from wild animals. The presence of AmpC producing bacteria in wild animals at pet market. This is the first study, to our knowledge, of enterobacteria harboring β-lactamase genes in wild animals in Saudi Arabia. The fact that these animals often live in close contact with their owners and other people in market make the occurrence of transmission between them even more likely. More studies should be carried out in the future in order to track the variants and evolution of β-lactamase genes compared to those from human isolates.

**Authors’ Contributions**

SAH conceived, designed the study, drafted and revised the manuscript. MYS collected and analyzed samples. Both authors read and approved the final manuscript.

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**Competing Interests**

The authors declare that they have no competing interests.

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