

Occurrence of carbapenem-resistant organisms among pet animals suffering from respiratory illness: a possible public health risk

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1. Abstract

The emergence of carbapenem-resistant organisms (CROs) has become a great challenge alarming the public health community. Thus, the current study was conducted to investigate the occurrence of CROs among pet animals suffering from respiratory illness. Nasal swabs from 100 pet animals (51 dogs and 49 cats) showing respiratory illness were screened for CROs. The obtained swabs were streaked onto CHROMagar mSuper CARBATM medium followed by sub-culturing on MacConkey agar. Colonies were identified by cultural characteristics, Gram staining, and biochemical tests as well as molecular techniques. Then, the identified isolates were confirmed to be CROs after being non-susceptible to at least one of four carbapenem antibiotics using the Kirby-Bauer method. The confirmed CRO isolates were also investigated on molecular basis for carbapenemase-encoding genes (*bla_{NDM}*, *bla_{KPC}*, *bla_{OXA-48}*, *bla_{VIM}*, *bla_{IMP}*). Out of 100 pet animals, 6 yielded CROs with an overall occurrence rate 6% (3.9% for all tested dogs and 8.2% for all tested cats). The obtained CRO isolates were *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae* with the following distribution among the examined animals 3%, 1%, 1%, and 1%, respectively. *bla_{NDM}* was the only detected carbapenemase-encoding gene among the isolates and it was detected in two cat isolates. In conclusion, the results of the current study highlight the emergence of carbapenem-resistant organisms among pet animals suffering from respiratory illness which may have a possible public health risk.

Key words: Carbapenem resistance; Carbapenemase gene; CRO; Pet animals; Respiratory illness.

2. Introduction

Throughout the life of pet animals, they may be subjected to different respiratory illnesses caused by bacterial or viral infections. Such illnesses may be manifested by coughing, pneumonia, noisy breathing, bronchitis, reduced ability to

exercise, changes in voice, and many more severe signs of breathing [1–4].

Many bacteria have been reported to cause bacterial respiratory infections among pets such as *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Bordetella bronchiseptica*, *Pasteurella* spp., and *Escherichia coli*. All these



bacteria circulated among companion animals and some of them have zoonotic potential considering the close contact between humans and pet animals gives a lot of opportunity for the transmission of these bacteria from the diseased pets to humans. In addition, some of such bacteria may be difficult to be controlled due to various factors of which the emergence of antimicrobial resistance (AMR) is the most important one [5–9].

The occurrence of AMR among bacterial pathogens has become a serious issue for both human and animal medicine. The presence of bacteria developing resistance to many antibiotics limits the drug options available for treatment [10, 11].

Carbapenems are β -lactam antibiotics used in human medicine as a last choice for severe cases and classified as ‘critically important’ for human use [12, 13]. Despite carbapenem antibiotics being classified as category A, which means avoiding its usage in animals except for specific cases, some reports pointed out the emergence of carbapenem-resistant organisms (CROs) among companion animals [14–16].

However, few studies have been conducted on this track. Therefore, the present study was carried out to investigate the occurrence of CROs among pet animals suffering from respiratory illness and highlight its public health implications.

3. Materials and Methods

Nasal swabs were collected from 100 pet animals exhibiting respiratory manifestations comprising 51 dogs and 49 cats. The obtained swabs were immediately transported to the laboratory in a transport medium.

3.1. Identification of carbapenem-resistant bacteria:

The collected swabs were plated onto CHROMagar mSuper CARBA™ agar (CHROMagar™, France), a selective chromogenic medium for isolation of CROs, and incubated aerobically overnight at 37°C [17]. Colonies with the characteristic red, metallic blue and green colors in addition to non-coloured ones were sub-cultured on MacConkey agar plates (Himedia, India) to obtain pure cultures. Isolates were fully identified using Gram staining, basic biochemical tests as well as API-20E test strips (BioMerieux, Marcy-l’Etoile, France). [18]

In addition, DNA from *Pseudomonas* spp. and *Klebsiella* spp. was extracted by boiling method according to Junior *et al.* [19] for molecular identification of *Pseudomonas aeruginosa* according to Al-Ahmadi and Roodsari [20] and *Klebsiella pneumoniae* according to Ebomah and Okoh [21].

3.2 Carbapenem resistance confirmation for the obtained bacterial isolates:

For all isolates, antibiotic susceptibility testing was done on Mueller-Hinton agar plates (Himedia, India) by the Kirby-Bauer disc diffusion technique as recommended by the Clinical and Laboratory Standards Institute (CLSI). The following carbapenems antibiotic discs were used: imipenem, meropenem, ertapenem, and doripenem. All breakpoints and results were recorded according to the CLSI guidelines [22].

3.3. Identification of carbapenemase-encoding genes among CRO isolates:

All CRO isolates were subjected to PCR for the detection of *bla*_{KPC}, *bla*_{IMP}, *bla*_{NDM}, *bla*_{VIM}, and *bla*_{OXA-48} genes coding for carbapenem-hydrolyzing enzymes.



Genomic DNA was extracted from the isolates via boiling method [19]. Five pairs of primers were used for the amplification of the genes of interest using a multiplex polymerase chain reaction (PCR) assay in 2 reactions (triplex and duplex) using the Cosmo PCR master mix. All primers and PCR conditions were carried out according to Li *et al.* [23]. PCR products were separated on 1.5% (w/v) agarose gel in an electrophoresis step to note the specific band for each gene

4. Results

The occurrence rate of CRO among 100 pet animals showing respiratory illness was 6% with 3.9 % in all dogs and 8.2 % in all cats as shown in table 1. The CRO isolates were identified as *E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*. The distribution of these isolates among the examined animals is shown in table 1.

All the six isolates showed resistance to one or more of the carbapenem drugs. The pattern of carbapenem susceptibility testing among the examined isolates is shown in table 2.

Regarding carbapenemase encoding genes, two feline isolates (*E. coli* and *Pseudomonas aeruginosa*) were harboring the *bla*_{NDM} gene (Table 3).

5. Discussion

Nowadays, the emergence of carbapenem resistance has been an important health challenge. In the current study, we shed more light on this issue among companion animals suffering from respiratory illness.

The results of the current study revealed that the occurrence rate of CROs among the examined animals was 6% with isolation rates of 3.9%, and 8.2% among all dogs and all cats, respectively. The

occurrence rate reported in this study is alarming considering that carbapenem drugs are not used in pet animals' medication. From our point of view, this may be due to the unauthorized misuse of carbapenem drugs for pet animals' medication as well as geographical distribution especially hospital and community settings. Few reports also stated rise in resistance to carbapenem in pet animals but there is scarce knowledge concerning respiratory carriage of CRO in pets. [24]

The prevalence rates of the identified bacteria were *E. coli* (3%), *K. pneumoniae* (1%), *P. aeruginosa*. (1%), and *E. cloacae*. (1%). Our results were supported by the findings of Stolle *et al.* [25] who isolated one *K. pneumoniae* and one *E. coli* with carbapenem resistance from broncho-alveolar lavage fluid take from a German shepherd dog with eosinophilic pneumonia. In addition, a study by Smith *et al.* [26] indicated that a *Pseudomonas* sp. isolate was found intermediately resistant to carbapenems, this isolate was recovered from a nasal swab of a dog with a history of complicated aspiration pneumonia in addition to an upper respiratory infection. Seriously, 4 out of the obtained 6 isolates including: 2 *E. coli*, one *Pseudomonas aeruginosa* and one *Enterobacter cloacae* were resistant to all carbapenem antibiotics to imply high level of carbapenem resistance.

Noteworthy, in this study, only *bla*_{NDM} of the five-targeted carbapenemase-encoding genes was detected in two feline isolates, one identified as *E. coli* and the other was *P. aeruginosa*. Similarly, Cole *et al.* [27] have detected five *E. coli* harbouring the *bla*_{NDM} gene from an endotracheal wash of 3 dogs; one from the lung tissue of a dog; and one from an endotracheal wash of a cat. High rates of bacteria harbouring the *bla*_{NDM} gene have



been reported in South Asia, the Balkans, North Africa and the Middle East, which may be linked to the population exchange with the Indian subcontinent where it was originally discovered [28].

To the best of our knowledge, this is the first record of *Pseudomonas aeruginosa* harbouring *bla*_{NDM} carbapenemase-encoding gene isolated from a cat with respiratory illness, whereas, *Pseudomonas* possessed *bla*_{VIM} or *bla*_{IMP} were previously reported among dogs with other diseases [29, 30].

It is worth mentioning that *Pseudomonas* sp. harbouring *bla*_{NDM} was recovered from wound samples collected from human clinics in Egypt and Libya [31,32]. Moreover, in India, Shanthi *et al.* [33] recovered four *P. aeruginosa* harbouring *bla*_{NDM} from sputum, endotracheal aspirate, pus and bronchoalveolar lavage from human patients in the chest ward and intensive care units. A matter which points out to the public health implication of *Pseudomonas aeruginosa* harbouring *bla*_{NDM}.

6. Conclusion

This study highlights the emergence of carbapenem-resistant organisms among pet dogs and cats suffering from respiratory illness with a potential zoonotic risk. Accordingly, appropriate bacteriological examination must be performed for pets suffering from respiratory illness to get the antibiotic of choice and combat such pathogens.

7. References

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Table 1: The occurrence of CROs among dogs and cats showing respiratory illness

Animals	Numbers tested	CRO isolates	Positive result No. (%)	Total No. (%)
Dogs	51	<i>E. coli</i>	1 (2%)	2 (3.9%)
		<i>Klebsiella pneumoniae</i>	1 (2%)	
Cats	49	<i>E. coli</i>	2 (4%)	4 (8.2%)
		<i>Pseudomonas aeruginosa</i>	1 (2%)	
		<i>Enterobacter cloacae</i>	1 (2%)	
Total	100			6 (6%)

Table 2: Carbapenem susceptibility testing of bacterial isolates recovered from dogs and cats with respiratory disorders

Animal species	CRO isolates	Carbapenem drugs' interpretation			
		Meropenem	Imipenem	Ertapenem	Doripenem
Dogs	<i>E. coli</i>	R*	R	R	R
	<i>Klebsiella pneumoniae</i>	S*	R	S	S
Cats	<i>E. coli</i>	I*	R	R	I
	<i>E. coli</i>	R	R	R	R
	<i>Enterobacter cloacae</i>	R	R	R	R
	<i>Pseudomonas aeruginosa</i>	R	R	R	R

* R: resistant I: intermediate S: susceptible

Table 3: Molecular identification of carbapenemase-encoding genes among bacterial isolates from respiratory ill dogs and cats

Animal species	Total number examined	CRO isolates	Genes	Number of positive	%
Dogs	51	0	0	0	0
Cats	49	<i>E. coli</i>	<i>bla</i> _{NDM}	1	2%
		<i>Pseudomonas aeruginosa</i>	<i>bla</i> _{NDM}	1	2%
Total	100			2	2%



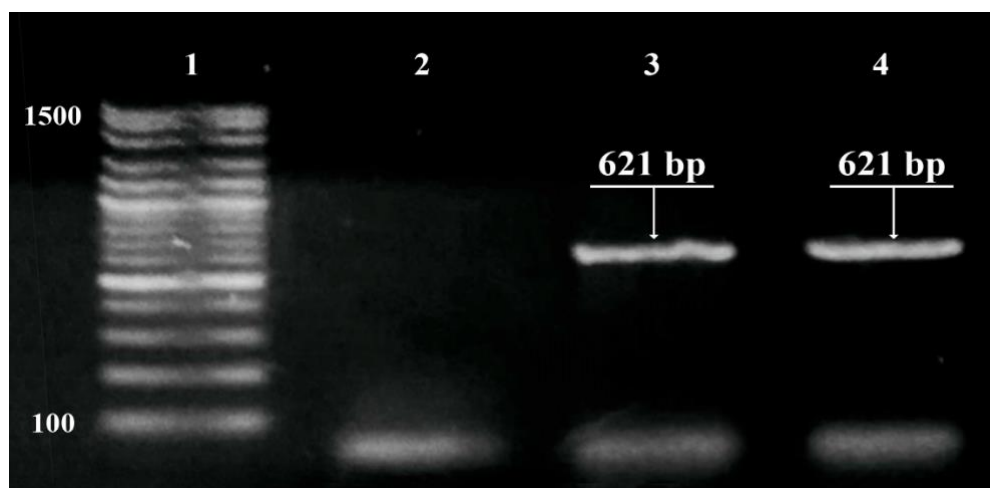


Fig. 1: Occurrence of *bla*_{NDM} among CROs recovered from respiratory ill dogs and cats. Lane 1: 100 bp DNA ladder; Lane 2: negative control (nuclease-free water); Lane 3 and 4: positive *bla*_{NDM} gene amplicons at 621 bp.

