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SHORT COMMUNICATION

Emergence of *bla*$_{NDM-5}$-producing *Escherichia coli* ST410 in Companion Dogs Treated with Meropenem

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A B S T R A C T

Carbapenem-resistant *Escherichia coli* (CRE) with a multidrug resistant phenotype was isolated from four clinically ill dogs treated with meropenem in different local animal hospitals between 2017 and 2019. IncX3-type plasmids of ca. 46 kb in size carrying *bla*$_{NDM-5}$ were present in all CRE strains and their transconjugants. High genetic similarity (>90%) by PFGE analysis was observed among the CRE strains, which were identified as ST410.To the best of our knowledge, *bla*$_{NDM-5}$-producing *E. coli* ST410 clones are emerging sporadically in companion dogs treated with meropenem. The spread of *Enterobacteriaceae* harboring the NDM-5 gene in companion animals can pose a threat to public health; therefore, extensive monitoring in veterinary hospitals using carbapenem and careful antibiotic use are crucial for managing and monitoring these resistant strains.

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INTRODUCTION

The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE), such as *E. coli*, *Klebsiella pneumoniae*, and *Citrobacter freundii*, which are important pathogens that cause human infections, is becoming an important issue for global health since they are resistant to almost all antibiotics. Global antimicrobial resistance has been a serious challenge to manage in hospitals due to rapid worldwide dissemination. Since New Delhi metallo-beta-lactamase-5 (NDM-5)-producing *E. coli* was first reported in 2011 (Hornsey *et al*., 2011), *bla*$_{NDM-5}$-producing *E. coli* clones in humans have gradually disseminated to Europe and Asia (Zhu *et al*., 2016 and Giufrè *et al*., 2018). In South Korea, NDM-5 and OXA-181-co-producing *E. coli* ST410 clones carrying IncFIA/B plasmid were reported in human patients (Baek *et al*., 2019). Recently, the first outbreak of NDM-5-producing *E. coli* harboring an IncX3-type plasmid in companion dogs with severe illness admitted to a local animal hospital was reported (Hong *et al*., 2019). Since then, clinical samples have been collected from companion animals to monitor the CRE. This study evaluated the clonality and plasmid transfer among carbapenem-resistant *E. coli* strains collected between 2017 and 2019.

MATERIALS AND METHODS

The laboratory was collected clinical samples from companion animals admitted to the veterinary hospital from July 2017 to June 2019 to investigate medically important resistant bacteria. Among them, carbapenem-resistant bacteria were isolated from companion dogs prescribed meropenem in four different animal hospitals in Seoul and Chungbuk. The clinical data of isolates are listed in Table 1.

The four carbapenem-resistant strains were identified using a microbial identification system (VITEK® MS, bioMérieux, Marcy-l'Étoile, France). Disk diffusion assays and the Sensititre standard susceptibility MIC plate ESB1F (TREK Diagnostic Systems/Thermo Fisher Scientific, Waltham, USA) test were performed to confirm resistance to antibiotics.

Multiplex PCR was performed to detect the carbapenemase genes, *bla*$_{NDM}$, *bla*$_{IMP}$, *bla*$_{OXA}$, *bla*$_{KPC}$, and *bla*$_{VIM}$, as described previously (Doyle *et al*., 2012). Seven housekeeping genes were amplified according to the protocols used for multilocus sequence typing (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). For pulsed-field gel electrophoresis (PFGE), plugs were prepared from the four CRE isolates and *Salmonella* Braendreup

H9812 as the size standard. Enzyme digestion by *Xba*I and electrophoresis by the CHEF Mapper XA system apparatus (Bio-Rad Laboratories, USA) were performed according to the CDC PulseNet standardized procedure (CDC PulseNet standardized procedure: https://www.cdc.gov/pulsenet/pdf/ecoli-shigella-salmonella-pfge-protocol-508c.pdf). DNA fragment patterns were compared using the Bionumerics program (Applied Maths, Kortrijk, Belgium).

In order to evaluate whether the plasmid-carrying NDM-5 in the CRE strains could be transferred to a recipient strain by conjugation experiment with *Escherichia coli* J53 which is resistant to sodium azide as a general recipient strain, plasmid replicon typing by PBRT Kit (https://www.diatheva.com/images/DATASHEET/MBK_MBR/MBK0078%20PBRT%202.0%20kit.pdf) and Southern hybridization (Rapley and Williams, 2002) were performed. Plasmid DNA was isolated from a wild strain EC17-33 carrying two plasmids that had been previously analyzed by whole genome sequencing (GenBank accession number, MH094148; IncX3-type plasmid of ca. 46 kb carrying $bla_{\text{NDM-5}}$; IncFIA/B type plasmid of ca. 74 kb carrying $bla_{\text{CTX-M-15}}$ and four transconjugants carrying the $bla_{\text{NDM-5}}$ gene using the High Pure Plasmid Isolation Kit (Roche Diagnostics) according to manufacturer's introduction.

RESULTSAND DISCUSSION

All strains were identified as *Escherichia coli*, which were resistant to carbapenem antibiotics (MIC to meropenem, > 8 mg/L; MIC to imipenem, 8 mg/L). These carbepenem-resistant *E. coli* (CRE) were resistant to all antibiotics tested, except for amikacin (Table 1). Carbapenemase gene screening revealed that all isolates were positive for the bla_{NDM} gene. All four CRE strains were typed as NDM-5 by sequencing analysis. The $bla_{\text{NDM-5}}$-producing multidrug resistant *E. coli* strains were identified as ST410 and could be identified as clones with a high degree of similarity (>90%) in dendrogram analysis by PFGE (Fig. 1). Despite their detection in different local animal hospitals, the isolate from 2017 and two isolates from 2018 were identical clones (cluster A1) with strong clonal relationships (cluster A2) to the 2019 isolate. One or two Inc plasmids, including IncX3, were identified in all transconjugants. Plasmid DNA was subjected to gel electrophoresis, capillary transfer onto a nylon membrane, and hybridization with NDM-5 gene probe, with all transconjugants confirming that the $bla_{\text{NDM-5}}$ gene belonged to the IncX3-type plasmid (Fig. 2).

Since multidrug-resistant NDM-5-producing *E. coli* ST410 carrying IncX3 plasmid was first discovered in four companion dogs admitted to local animal hospital in Seoul, 2017 (Hong *et al*., 2019), three further CRE organisms have been detected in three different local animal hospitals(2 in Seoul and 1 in Chungbuk) between 2018 and 2019. The majority of the CRE strains were isolated from the nasal cavity, urine, or stools of companion dogs with severe infectious diseases, such as pneumonia, chronic bronchitis, and cystitis, after meropenem prescription. In this study, all four CRE strains were confirmed to be identical clones using molecular epidemiological data and harbored IncX3 plasmids with NDM-5 gene. Consequently, it was confirmed that multidrug-resistant NDM-5-producing *E. coli* ST410 clones carrying IncX3 plasmid had sporadically emerged in 4 different animal hospitals. These CRE clones have never been reported in human patients in South Korea. In other words, plasmid replicon types identified among *E. coli* ST410 strains with NDM-5 between humans and companion animals were found to be different and their epidemiological association was also confirmed to be low.

Recently, epidemic NDM-5-producing *E. coli* harboring IncX3 plasmid has been reported in China, United Arab Emirates, and Czech Republic, although the sequence types (STs) are different, and is mostly found in human patients (Mouftah *et al*., 2019). The size and resistance genotypes of IncX3 plasmids carrying NDM-5 in these CRE strains were different patterns from the plasmids identified in this study. However, the genetic structure and the size of IncX3 plasmid harboring NDM-5 in this study was identical to that of *E. coli* and *Proteus mirabilis* clinical isolates from Chinese patients in 2013 and 2018, respectively (Zhu *et al*., 2016 and Sun *et al*., 2019). Plasmids of the same genetic structure have been identified in different *Enterobacteriaceae* strains in neighboring country; however, it was difficult to trace the epidemiology of these plasmids. Nevertheless, our data suggest that IncX-type plasmids have the potential to be transferred to other organisms. Moreover, we cannot exclude that the abuse of carbapenem antibiotics in companion animals may trigger carbapenem resistance, since NDM-5-producing bacteria have emerged in companion animals treated with meropenem.

Conclusions: *Enterobacteriaceae* harboring IncX3 plasmid carrying $bla_{\text{NDM-5}}$ in companion animals could be threat to public health; therefore, it is necessary to monitor the emergence of carbapenem-resistant bacteria.

Table 1: Characteristics of four $bla_{\text{NDM-5}}$-producing *Escherichia coli* isolates from clinically ill dogs

Isolation year	Strain no.	Disease	Province	Specimen	Medication	Prognosis	Antimicrobial resistance profiles
2017	EC033	Pneumonia, CHF	Seoul	Stool	Meropenem	Death	AMP-AMC-SAM-CFM-CTX-CAZ-FOX-FEP-MEM-IPM-CIP-SXT
2018	EC020	Cancer, Cystitis	Seoul	Urine	Amoxicillin/clavulanate, Meropenem	Death	AMP-AMC-SAM-CFM-CTX-CAZ-FOX-FEP-MEM-IPM-CIP-GEN-SXT
2018	EC211	Cystitis	Chungbuk	Urine	Amoxicillin/clavulanate, Meropenem	Recovery	AMP-AMC-SAM-CFM-CTX-CAZ-FOX-FEP-MEM-IPM-CIP-GEN-SXT
2019	EC011	Chronic bronchitis	Seoul	Nasal cavity	Doxycycline, Meropenem	Recovery	AMP-AMC-SAM-CFM-CTX-CAZ-FOX-FEP-MEM-IPM-CIP-GEN-SXT

Disease: CHF, congestive heart failure. Antibiotics: AMP, ampicillin; PIP, piperacillin; AMC, amoxicillin-clavulanate; SAM, ampicillin-sulbactam; CFM, cefixime, CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; MEM, meropenem; IMP, imipenem; TET, tetracycline; CIP, ciprofloxacin; GEN, gentamicin; SXT, trimethoprim-sulfamethoxazole.

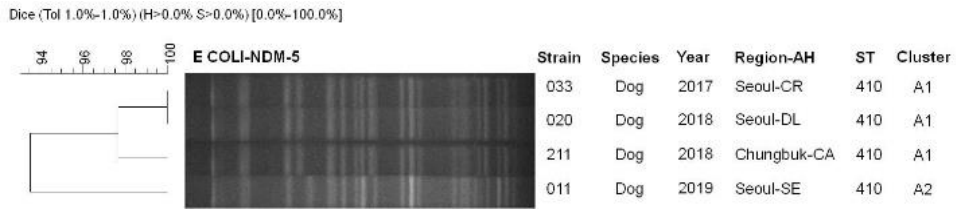

Fig. 1: Dendrogram of 4 PFGE profiles from *Xba*I-PFGE of carbapenem-resistant *Escherichia coli* strains harboring *bla*$_{NDM-5}$ isolated from clinically ill dogs between 2017 and 2019 in South Korea.

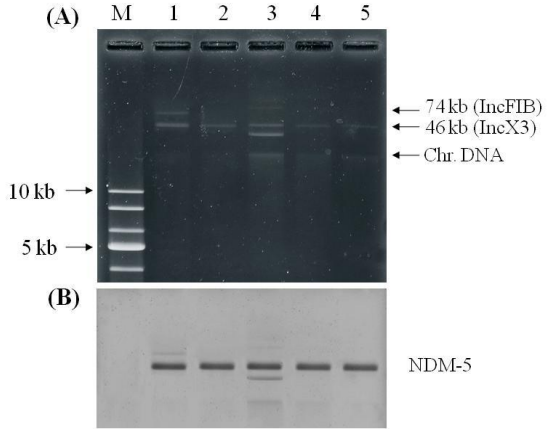

Fig. 2: (A) Plasmid DNA extracted from a wild strain and four transconjugants. (B) Southern hybridization with *bla*$_{NDM-5}$. Lanes: 1, *Escherichia coli* wild type strain EC033 harboring *bla*$_{NDM-5}$; 2, pJC033; 3, pJC020; 4, pJC211; 5, pJC011; M, 1 kb DNA ladder marker.

Authors contribution: JY and S executed all experiments and WK and JC analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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