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# Community-acquired Extended-spectrum and Plasmid-mediated *amp*C Beta-lactamase-producing Multidrug-resistant *Enterobacter cloacae* Septicaemia in a Cat with Euthyroid Sick Syndrome

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**Abstract :** A 7-year-old castrated male Korean Shorthair cat was referred with lethargy and anorexia. Laboratory examination revealed moderate degenerative changes of peripheral neutrophils on blood smear examination and decreased levels of free and total thyroxine ($T_4$) as well as bacterial growth on blood culture. Molecular analyses of the 16S ribosomal RNA gene and heat shock protein 60 gene confirmed the bacterium as *Enterobacter cloacae*. A minimal inhibitory concentration test showed multidrug resistance of the bacterium against 16 antibiotics. Polymerase chain reaction (PCR) and subsequent sequencing specifically for $bla_{TEM}$, $bla_{SHV}$, $bla_{CTX-M}$, and plasmid-mediated *amp*C genes revealed positive results to $bla_{TEM-1}$, $bla_{CTX-M-15}$, and plasmid-mediated $bla_{ACT-1}$ genes, indicating that the isolated bacterium contains plasmids containing genes encoding extended-spectrum beta-lactamase and plasmid-mediated *amp*C beta-lactamase. After 1 month of treatment with antibiotics and levothyroxine, the cat's condition improved; both the thyroid function test and the blood culture showed no abnormalities. This is the first report of community-acquired multidrug-resistant *E. cloacae*-induced euthyroid sick syndrome in a cat. By the prompt diagnostic procedures and properly selected antibiotic therapy, the cat was recovered from the multidrug-resistant bacterium-induced septicaemia.

***Key words*** **:** cat, septicaemia, *Enterobacter cloacae*, multidrug resistance, euthyroid sick syndrome.

## Introduction

*Enterobacter cloacae* is a member of the normal gastrointestinal flora in both humans and animals, and it is also widely distributed in the environment (5,20). In humans, *E. cloacae* is an important hospital-associated pathogen responsible for urinary tract infection, bacteraemia, pneumonia, endocarditis, septic arthritis, osteomyelitis, and skin and soft tissue infections (8,16). Recently, multidrug resistance of these organisms has become a concern, particularly with the emergence of extended-spectrum beta-lactamase (ESBL) and *amp*C beta-lactamase producing strains (9,18). The number of dogs with multidrug resistance has also increased, possibly because of the popular use of intensive care units, longer hospitalization periods, and introduction of a large number of new antibiotics.

Euthyroid sick syndrome refers to the suppression of serum thyroid hormone concentrations in response to concurrent illness (3). Euthyroid sick syndrome has been established in both humans and dogs; however, serum thyroid hormone concentrations are also altered in cats as a result of a variety of nonthyroidal diseases, and there is a direct correlation between the severity of illness and the magnitude of the decrease in thyroid hormone concentration (11,13,15). Chronic renal failure, diabetes mellitus, neoplasia, gastrointestinal disorders, primary hepatic disease, hypertrophic cardiomyopathy, respiratory disease, and neurologic disease have been described as causes of euthyroid sick syndrome in cats.

This report describes a case of euthyroid sick syndrome in a free-ranging cat, provoked by systemic infection of multidrug-resistant (MDR) *Enterobacter cloacae.* The diagnosis was made by blood culture, bacterial identification, minimal inhibitory concentration (MIC) test, thyroid hormone assays, and therapeutic trials. To our knowledge, this is the first case report of euthyroid sick syndrome caused by MDR *E. cloacae* in an adult cat.

## Case

A 7-year-old castrated male Korean Shorthair cat, weighing 5.6 kg, presented with lethargy and poor appetite. The symptoms were noticed 1 week earlier by the owner and were thought to have slowly progressed. Before symptoms onset, the cat had never been prescribed medication or vaccinations and had only been neutered. The patient was a free-ranging cat living in a temple located downtown and was fed commercial dry cat food. On physical examination, the cat was slightly obese, and his coat was dull and matted. No other abnormalities, except less sensitivity to external stimuli, were found.

Laboratory examinations including complete blood count (CBC), serum biochemistry profiles, electrolytes, and urinalysis showed no abnormalities. On blood smear examination, Döhle bodies and cytoplasmic vacuolation were frequently observed in the cytoplasm of neutrophils, indicating a moder-

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**Table 1.** Test results of free and total thyroxine ($T_4$) concentrations in the cat

| Item | Value (ng/dL) | Reference interval (ng/dL) |
|---|---|---|
| Free $T_4$ | < 0.3 | 1.00-2.50 |
| Total $T_4$ | < 0.5 | 1.00-4.00 |

ate level of degenerative changes in neutrophils. Radiographic and ultrasonographic examinations of the abdomen and thorax showed no remarkable findings. As the cat was a free-ranging animal with no vaccinations, in-house feline antigen-capturing enzyme-linked immunosorbent assay (Ag-ELISA) tests for feline immunodeficiency virus, feline leukaemia virus, and feline parvovirus (SNAP® FIV/FeLV Combo test, IDEXX, USA; Anigen Rapid FPV Ag test, BioNote, Korea) were performed and confirmed to be negative. Peripheral blood and stool were submitted to a referral diagnostic laboratory (Neodin Vetlab, Korea) for polymerase chain reaction (PCR) assays to test for the following pathogens: *Babesia* spp., *Ehrlichia* spp., *Haemobartonella* spp., feline leukaemia virus, feline coronavirus, feline panleukopenia virus, feline herpes virus, feline calicivirus, feline immunodeficiency virus, *Chlamydia* spp., *Toxoplasma gondii*, *Anaplasma phagocytophilum*, and *Borrelia burgdorferi*; the cat was confirmed negative for all of these pathogens. Gastrointestinal endoscopy and computed tomography under general anaesthesia showed mild edema of gastric mucosa, which was confirmed as benign non-inflammatory mucosal hypertrophy in histopathologic examination.

The cat was further tested for thyroid dysfunction and blood culture. Tests for free and total thyroxine ($T_4$) revealed values below normal reference intervals (Table 1), indicating the possibility of hypothyroidism or euthyroid sick syndrome. The blood cultures under aerobic and anaerobic conditions showed bacterial growth in both conditions (Fig 1). The fluids from the culture bottle were subcultured aerobically or anaerobically on trypticase soy agar with 5% sheep blood at 37°C for 24 h, and the resultant colonies were tested for bacterial identification. Following DNA extraction using a DNA mini kit (Qiagen, Valencia, CA, USA), the 16S ribosomal RNA gene (16S rDNA) and heat shock protein 60 (*hsp*60) gene of both isolates were amplified and sequenced using a pair of 27F and 1492R primers (for 16S rDNA) and a cocktail of h279, h280, h1612, and h1613 (for *hsp*60) as previously described (4,7). Compared with the sequences in the GenBank database, the deduced nucleotide sequences of both isolates were found to be 99% similar to the 16S rDNA or *hsp60* gene sequence of *E. cloacae* that had been deposited by the Hisashi Nishiwaki Kinki University in Japan or by the Sanger Institute in the United Kingdom (GenBank accession numbers AB244457 for 16S rDNA or FP929040 for *hsp*60; Fig 2).


**Fig 1.** Aerobic (blue-labeled bottle) and anaerobic (red-labeled bottle) blood culture showed opaque culture fluid in the bottle, indicating bacterial growth.

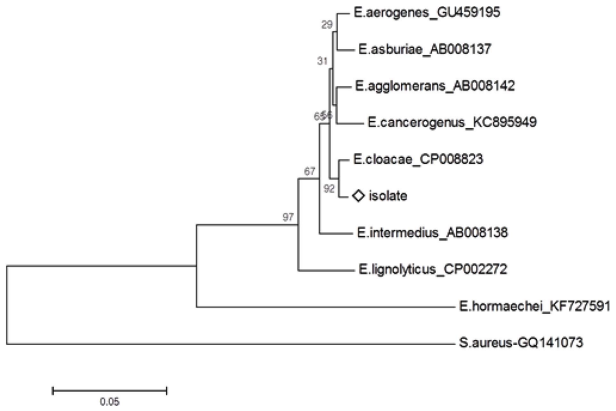


**Fig 2.** Neighbour-joining phylogenetic tree based on the alignment of heat shock protein 60 (*hsp*60) gene sequences of *Enterobacter* spp. The diamond indicates the sequence of *E. cloacae* isolated from the cat. The *hsp*60 sequence of *Staphylococcus aureus* was selected as an out-group. Sequence alignments were performed using BioEdit v.7.0.5.3, and MEGA6 v.6.06 was used for phylogenetic analysis. Bootstrap values are shown on the branches. The number following the species name indicates the GenBank accession number of each *Enterobacter* spp.

The MIC of isolated *E. cloacae* determined by the broth microdilution method with the Sensititre plates EUST and COMPN1F (TREK Diagnostic Systems, Cleveland, OH, USA) showed that the isolated bacteria was resistant to 16 antibiotics (tetracycline, chloramphenicol, kanamycin, gentamicin, trimethoprim, ciprofloxacin, cefoxitin, sulfamethoxazole, ampicilin, amoxicillin/clavulanate, ticarcillin, trimethoprim/sulfamethoxazole, cefpodoxime, ticarcillin/clavulanate, doxycyline, and cefazoline), indicating multidrug resistance (Table 2).

Since the isolated bacteria was resistant to cefpodoxime, it was further tested for ESBL and *amp*C beta-lactamase production via double disc synergy test (DDST) with discs of cefotaxime and amoxicillin/clavulanate (Oxoid, Hampshire, United Kingdom) and PCRs specific for $bla_{TEM}$, $bla_{SHV}$, $bla_{CTX-M}$, and plasmid-mediated *amp*C genes as previously described (2,12,17). While the discs of the DDST could not suppress the growth of isolated bacteria, PCRs were positive for the $bla_{TEM}$, $bla_{CTX-M}$, and $bla_{ACT-1}$ genes (Fig 3). After se-

**Table 2.** Antibiotic resistance profiles of the isolated *Enterobacter cloacae* in this study

| Antibiotic | Test range (mg/L) | Resistance breakpoint | Test result |
|---|---|---|---|
| Tetracycline | 0.5-16 | ≥ 16 | > 16 |
| Chloramphenicol | 4-64 | ≥ 17 | > 64 |
| Kanamycin | 4-64 | ≥ 25 | 32 |
| Gentamicin | 1-16 | ≥ 8 | > 16 |
| Trimethoprim | 2-32 | ≥ 16 | > 32 |
| Ciprofloxacin | 0.25-8 | ≥ 4 | > 8 |
| Cefoxitin | 0.5-16 | ≥ 32 | > 16 |
| Sulfamethoxazole | 64-512 | ≥ 350 | > 512 |
| Ampicillin | 0.25-16 | ≥ 32 | > 16 |
| Amoxicillin/ clavulanate | 4/2-32/16 | ≥ 32/16 | > 32/16 |
| Ticarcillin | 8-64 | ≥ 128 | > 4 |
| Trimethoprim/ sulfamethoxazole | 0.5/9.5-2/38 | ≥ 2* | > 2/38 |
| Amikacin | 4-32 | ≥ 32 | < 4 |
| Cefpodoxime | 2-16 | ≥ 8 | > 16 |
| Imipenem | 1-8 | ≥ 16 | 2 |
| Ticarcillin/clavulanate | 8/2-64/2 | ≥ 128/2 | > 64/2 |
| Doxycycline | 2-8 | ≥ 16 | > 8 |
| Cefazolin | 4-16 | ≥ 32 | > 16 |

*Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoint is expressed as the trimethoprim concentration.

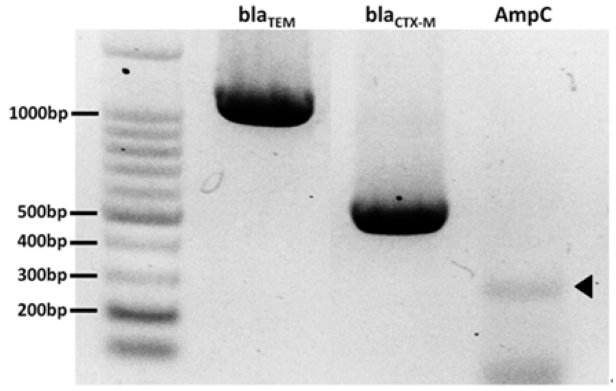


**Fig 3.** Expression of $bla_{TEM}$, $bla_{CTX-M}$, and $bla_{ACT-1}$ genes in the target gene-specific PCR for *E. cloacae* isolate. All amplicons were confirmed as their corresponding subtypes by subsequent sequencing and sequence analyses.

quencing of the amplicons, the deduced nucleotide sequences were identical to $bla_{TEM-1}$, $bla_{CTX-M-15}$, and plasmid-mediated $bla_{ACT-1}$ genes, indicating that the isolated bacteria contains plasmids containing genes that encode for ESBL and plasmid-mediated *amp*C beta-lactamase. Thus, the case was tentatively diagnosed as ESBL and *amp*C beta-lactamase-producing MRD *E. cloacae* septicaemia causing euthyroid sick syndrome.

In the meantime, the cat was hospitalized and treated with intravenous crystalloid fluid with 5% glucose and nasogastric tube feeding as well as a combination of intravaneous antibiotics (ampicilin/sulbactam: 20 mg/kg and cefotaxime: 30 mg/kg), daily oral antibiotics (cefixime: 12.5 mg/kg and ciprofloxaxin: 15 mg/kg) and levothyroxine (10 μg/kg) as initial therapy. After the MIC test, ciprofloxacin was substituted by daily intravaneous injection of amikacin (20 mg/kg). During 1 month of hospitalization, the cat started to display an interest in food after approximately 2 weeks of amikacin treatment, and his activity level slightly improved. The thyroid profiles and blood culture were rechecked and showed values within the reference interval and were negative for the bacterial growth, respectively. Based on the owner's decision, the cat was discharged from the hospital and prescribed the same dosage of oral antibiotic (cefixime) for another month. During the phone call with the owner after the month, the cat was confirmed to return to his daily life; however, follow-up examination could not be performed because the owner did not want to visit the hospital again.

## Discussion

This report describes a case of bacterial septicaemia causing euthyroid sick syndrome, which was confirmed by blood culture, antibiotic susceptibility testing, thyroid profiles, and therapeutic trials. The bacterial identification and MIC test identified the isolated bacteria as MDR *E. cloacae*. Interestingly, the resistant gene-specific PCR and sequencing confirmed that the isolated bacteria concurrently contained 3 types of plasmid-mediated beta-lactamases (TEM-1, CTX-M-15, and *amp*C), even though the cat was free-ranging and not hospitalized or even treated before he presented with *E. cloacae* infection. Considering the fact that the cat was housed in a temple located downtown, it is presumed that the route of transmission for the community-acquired plasmid-mediated antibiotic resistance might be a result of contact with temple worshippers, as the cat was continuously exposed to these people and directly touched or took food from them.

In this case, the isolated bacteria was first suspected to produce ESBL based on the resistance to cefpodoxime; however, in the DDST, clavulanate in combination with cefotaxime did not suppress the bacterial growth. The clinical microbiological tests that are used for detecting ESBLs employ a beta lactamase inhibitor (i.e., clavulanate) in combination with ceftazidime or cefotaxime, which are third-generation cephalosporins (1). However, previous reports indicated that clavulanate could not inhibit some gram-negative bacteria with coexpression of both ESBLs and AmpC, because in such situations, clavulanate induces the expression of *amp*C beta-lactamase that acts as cephalosporinase, resulting in a false-negative result (10,19). Recently, a modified DDST with cefepime, a fourth-generation cephalosporin, showed higher diagnostic sensitivity than that with cefotaxime or ceftazidime (5). In this report, the isolate was not suppressed by DDST with cefotaxime and clavulanate, even though the isolate expressed 2 types of ESBLs (TEM-1 and CTX-M-15) probably because of the expression of ACT-1, one of *amp*C beta-lactamase. Our observation indicates that the modified DDST with the fourth-generation cephalosporin and beta-lactamase inhibitor is necessary to achieve accurate diagnosis and epidemiologic study for ESBL-producing bacteria of animal origin.

Regardless of its aetiology, accurate diagnosis of euthyroid sick syndrome in cats can be challenging, because none of its pathognomonic changes -i.e. clinical signs, physical examination, and routine laboratory test findings- are necessarily unique to euthyroid sick syndrome (14). The current recommendation for both diagnosis of and differentiation between hypothyroidism and euthyroid sick syndrome in cats is rechecking the patient for nonthyroidal illness, even if the total and free $T_4$ simultaneously decrease, because severe systemic illness can induce decrease of both forms of $T_4$ (3). In this report, the cat did not show any of pathologic changes upon laboratory examination, including CBC, serum biochemistry profiles, and electrolytes; thus, the case was nearly misdiagnosed as true hypothyroidism, especially since the cat was negative for all blood-borne viral/rickettssial pathogens based on Ag-ELISAs and PCRs. As final confirmation tests, blood culture with molecular analyses for bacterial pathogens revealed *E. cloacae*-induced septicaemia, and a final diagnosis of septicaemia-induced euthyroid sick syndrome was made by therapeutic trials. To the best of our knowledge, this is the first report that describes a case of *E. cloacae*-induced euthyroid sick syndrome in a cat. We recommend that small animal clinicians pay careful attention to cats with suspected hypothyroidism to identify possible bacterial infection that can provoke euthyroid sick syndrome.

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# 정상 갑상샘 질환 증후군 고양이의 지역사회획득 광범위 및 플라스미드 유래 *amp*C beta-lactamase 양성 다약제내성 *Enterobacter cloacae* 패혈증

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**요 약** : 7년령, 중성화 수컷 고양이가 무기력과 식욕부진으로 내원하였다. 실험실 검사는 말초혈액 호중구에서 중등도의 퇴행성 변화, free 및 total thyroxine의 농도 감소 및 혈액배양에서 세균증식이 관찰되었다. 16S ribosomal RNA 및 heat shock protein 60 유전자 검사 결과 세균은 *Enterobacter cloacae*로 확인되었다. 항생제 최소억제농도 평가 결과 16개 항생제에 대한 다약제 내성이 관찰되었다. 중합효소연쇄반응 및 시퀀싱 결과 $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-15}}$ 및 플라스미드 유래 $bla_{\text{ACT-1}}$ 유전자에 대한 양성 반응이 관찰되었으며, 결과적으로 확인된 세균이 광범위 beta-lactamase 및 *amp*C beta-lactamase를 합성하는 플라스미드를 보유한 것을 확인하였다. 환자는 1개월 간 항생제와 levothyroxine 치료를 받은 후 증상이 호전되었고, 치료 후 갑상선 기능검사와 혈액배양 결과 이상소견은 소실되었다. 이 증례는 고양이의 지역사회획득 다약제내성 *E. cloacae*에 의한 정상 갑상샘 질환 증후군의 첫 예이다. 신속한 진단과정과 적절한 항생제 선택에 의해 이환된 고양이는 패혈증으로부터 회복되었다.

**주요어** : 고양이, 패혈증, *Enterobacter cloacae*, 다약제내성, 정상 갑상샘 질환 증후군