Case Report

Klebsiella pneumoniae carrying bla_{NDM-1} gene in orthopedic practice

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Abstract

Emergence and spread of carbapenemases in Enterobacteriaceae is a cause of concern worldwide, the latest threat being New Delhi metallo-β-lactamase (NDM-1). This report is of an orthopedic case with fracture femur managed with internal fixation and bone grafting, who subsequently developed secondary infection with *Klebsiella pneumoniae* harboring bla_{NDM-1} gene. Minimum inhibitory concentration (MIC) of imipenem was ≥8 μg/ml by E-test, suggestive of carbapenemase production. Phenotypic and further genotypic detection confirmed the presence of bla_{NDM-1} gene. The isolate remained susceptible only to tigecycline, colistin, and polymyxin B.

Key words: Carbapenemase, *Klebsiella pneumoniae*, New Delhi metallo-β-lactamase
MeSH terms: Klebsiella, orthopedic surgery, betalactamase

Introduction

Resistance to antimicrobials and a paucity of new antimicrobial agents are ongoing challenges, more so in orthopaedic surgery. Carbapenems are one of the very few therapeutic agents available for treating multidrug resistant infections caused by Enterobacteriaceae; however, carbapenemases are increasingly being reported, with the latest threat being New Delhi metallo-β-lactamase (NDM-1). NDM-1 was initially reported in *Klebsiella pneumoniae* and *Escherichia coli* in a Swedish patient of Indian origin,[1] and several published articles, particularly case reports, show the presence of NDM-1 containing pan-resistant Enterobacteriaceae mainly from the Indian subcontinent.[2-6] However, now NDM-1 carriage has spread in enterobacterial isolates the world over, which is a matter of great concern.[7] This is the report of an orthopedic case with fracture femur managed with internal fixation and bone grafting, who subsequently developed secondary infection with *K. pneumoniae* harboring bla_{NDM-1} gene.

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Case Report

A 32-year-old male patient, presented with a Grade IIIA open fracture of the right distal femur, following a roadside accident suffered 22 hours back. Local examination revealed two (sutured) lacerated wounds on the distal one-third of the anterior and lateral aspects of the right thigh. There were no other injuries, and the distal neurovascular function was intact.

After stabilization of the patient, he was taken up for surgery after 24 hours of admission (46 hours of injury). Through a lateral surgical approach (incorporating the existing wounds), the wounds were debrided, intercondylar fracture was stabilized with K wires and lag screws, and the distal femur fracture was stabilized with a locking plate. At the time of surgery, there was significant bone loss from the lateral and posterior parts of the distal femur, but bone grafting was not done due to the fear of infection in the contaminated wound which was being operated after a delay of 46 h. Wound was closed on a negative suction drain. The patient was put on gentamicin and cefoperazone/sulbactum. One week later, the wound was apparently healthy, so the patient underwent bone grafting at the fracture site. Intraoperatively, fluid and tissue from the fracture site were sent for culture, which came out to be sterile.

On the third day of the second surgery, the wound showed seropurulent discharge which on culture/sensitivity yielded pure growth of multidrug resistant *K. pneumoniae*.

Since the organism was highly resistant, the patient was put on amikacin (1 g once daily), high-dose imipenem (1 g 8-hourly as infusion in normal saline over 2 h), and rifampicin (600 mg once daily). A repeat pus culture after 2 days again revealed the same organism. A decision for wound debridement and removal of bone graft was made and performed which resulted in healing of the wound. No further colonization with the same bacterium was detected by taking rectal swabs from that patient or from other patients hospitalized simultaneously in the same unit.

In the laboratory, *K. pneumoniae* isolate was found to be multidrug resistant by disk diffusion method as per the Clinical Laboratory Standards Institute (CLSI) 2011 guidelines.[8] It was resistant to ceftazidime, cefotaxime, cefipime, gentamicin, amikacin, ciprofloxacin, aztreonam, amoxicillin/clavulanic acid, cefoperazone/sulbactam, piperacillin/tazobactam, ampicillin/sulbactam, cefipime/tazobactam, ertapenem, meropenem, doripenem, and imipenem. We screened the isolate for extended-spectrum β-lactamase (ESBL) production by disk potentiation test, but it was found to be negative. Evaluation of the isolate for ESBL using E-test strips containing ceftazidime at one end and ceftazidime–clavulanic acid at the other end, as well as Amp C β-lactamase by using E-test strips containing cefotetan at one end and cefotetan/cloxacillin at the other end was also negative. Minimum inhibitory concentration (MIC) of imipenem was ≥8 μg/ml by E-test, suggestive of carbapenemase production. Modified Hodge test on the isolate was negative, ruling out class A carbapenemases and pointing toward metallo-β-lactamases (MBLs). Phenotypic method with disk synergy test using imipenem and ethylenediaminetetraacetic acid (EDTA) and further genotypic detection confirmed the presence of bla_{NDM-1} gene [Figure 1] in hospital-acquired *K. pneumoniae* isolate, conferring resistance to carbapenems including doripenem. The isolate remained susceptible only to tigecycline (MIC ≤1.5 μg/ml), colistin (MIC ≤1 μg/ml), and polymyxin B (MIC ≤1 μg/ml).

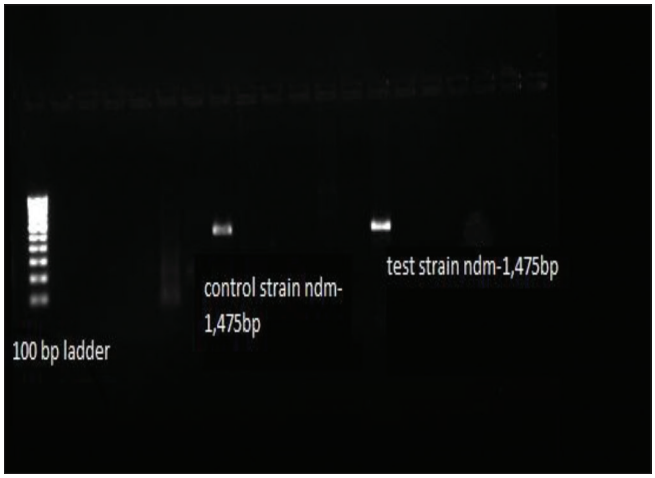

Figure 1: Pulsed-field gel electrophoresis pattern of NDM-1 producing *Klebsiella pneumoniae*

Molecular analysis technique

DNA was extracted from the strain by heat boil method, and the DNA was subjected to single-target polymerase chain reaction (PCR). Two microliters of the extracted total DNA was subjected to PCR in a 50-μl reaction mixture. The PCR mixture for the detection of MBL genes contained 1 × PCR buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl], 1.5 mM MgCl2, 0.125 mM of each deoxynucleotide triphosphate, 0.1 μM of each primer, and 2 U of DNA polymerase. The primers used were F**:** 5′-GGGCAGTCGCTTCCAACGGT-3′ and R: 5′-GTAGTGCTCAGTGTCGGCAT-3′, which amplified an internal fragment of 475 bp for NDM-1 gene. Amplification was carried out under the following thermal cycling conditions: 10 min at 94°C; 36 cycles of amplification consisting of 30 s at 94°C, 40 s at 52°C, and 50 s at 72°C; and 5 min at 72°C for the final extension. Amplified products (475 bp) from the test strain and control strain were visualized under UV light on 3% agarose gel electrophoresis [Figure 1].[9]

Discussion

Acquired carbapenemases confer extensive antibiotic resistance in Enterobacteriaceae and represent a public health threat. A novel acquired carbapenemase, NDM-1, has now been described from several nations worldwide,[7] and was earlier being reported mostly in patients who had received treatment in the Indian subcontinent.[2-6] Like other acquired MBLs, NDM-1 hydrolyzes all β-lactam antibiotics except aztreonam.

NDM-1, named after the city of origin (New Delhi) and which has been recently criticized, is normally carried on a variety of plasmids along with genes conferring resistance to almost all other antibiotics. This enzyme shares very little identity with other MBLs, with the most similar MBLs being VIM-1/VIM-2, with which it has only 32.4% identity.[1] Most plasmids detected in these clinically relevant bacteria are easily transferable and capable of wide rearrangement, suggesting rapid and global dissemination and malleability among bacterial populations.[3] An association with other resistance mechanisms makes a majority of Enterobacteriaceae with bla_{NDM-1} extensively resistant to antibiotics and susceptible only to polymyxins and less consistently to tigecycline; similar was the finding in our isolate.

K. pneumoniae is a well known hospital-acquired pathogen, notorious for being multidrug resistant. Castanheira *et al.*[10] reported that isolates producing NDM-1 were disseminated in Indian health care facilities as early as 2006, but reports of a clinical association of *K. pneumoniae* carrying bla_{NDM-1} gene responsible for wound infections are rare. Recently there has been a report

on diabetic foot ulcer patients.[11] To treat these infections, combining drugs from different classes of antibiotics is to be resorted to or the pus/foreign agent should be removed as was done in the present case.

Invasive infections by carbapenem-resistant strains are associated with high morbidity and mortality rates and such case reports alert the surgeons and physicians about the threat of such multidrug-resistant strains. To conclude, infections by carbapenem-resistant bacteria are difficult to treat and rapid identification of MBL-producing Gram-negative species is crucial both for appropriate treatment and for timely implementation of infection control measures.

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