Various combinations of Carbapenemase production in community-acquired clinical isolates in Nepal

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Abstract

Background Carbapenems are at the forefront of managing infections caused by multidrug-resistant organisms (MDROs). These antibiotics are destroyed by carbapenemase enzymes, produced by the infecting bacteria. Klebsiella pneumoniae Carbapenemase (KPC), New Delhi Metallo-β-lactamase (NDM), Verona integron-encoded Metallo-β-lactamase (VIM), OXA-48, and IMP are the commonest carbapenemases reported. Since these resistance phenotypes are related to hospitals, their place in the community is actually unclear and unexpected. Our case series is, therefore, crucial to reveal the significant production of multiple carbapenemases in these alarmingly resistant community-acquired isolates recovered from three patients.

Case presentation We confirmed carbapenemase production by using the modified Hodge test (MHT) and Xpert Carba R. NDM, KPC and OXA-48 carbapenemase production, VIM, NDM, KPC, and OXA carbapenemase production, and IMP, VIM, NDM, and OXA carbapenemase production were demonstrated in Case 1, Case 2, and Case 3, respectively. Escherichia coli was isolated from blood and urine samples of case 1. The isolate was susceptible to colistin, tigecycline, and fosfomycin. Due to the development of acute kidney injury, treatment with colistin combined with tigecycline was stopped and Inj. Fosfomycin was started intravenously. After completion of antimicrobial therapy, the patient successfully recovered. Blood and sputum cultures of Case 2 with community-acquired pneumonia, yielded multidrug-resistant Klebsiella pneumoniae. The strain was carbapenemase producer and susceptible to tigecycline and colistin solely. Treatment with intravenous colistin and tigecycline resulted in a good clinical outcome. E. coli was recovered from both the ascitic fluid and the blood of Case 3. The pathogen was susceptible to both colistin and tigecycline. After the modification of treatment modality from intravenous imipenem to tigecycline, the patient began to improve on the third day of therapy.

Conclusions Our results are noteworthy in emphasizing the growing resistance profiles of gram-negative bacteria in the community and show an epidemiological link between healthcare settings and the community. These bacteria are easily transmissible by mobile genetic elements (MGEs), compelling us to rely on last-resort drugs like colistin, tigecycline, or fosfomycin for better therapeutic
management. Accordingly, we used these drugs in combination, and all three patients were cured.

Introduction
An increase in antimicrobial resistance (AMR) has become a common and critical health issue, crippling the healthcare system both clinically and socioeconomically. Multidrug-resistant (MDR) gram-negative organisms top the list of difficult to treat infections, and carbapenems have become the last resort antibiotics. Increased use of carbapenems has led to the emergence of carbapenem-resistant organisms (CRO). Treatment options for carbapenem-resistant Enterobacteriaceae (CRE) are limited to polymyxins, tigecycline, fosfomycin, and aminoglycosides. These antibiotics have pharmacokinetic (PK) and pharmacodynamic (PD) limitations obscuring their use [1]. Resistance to carbapenems can come up by the production of carbapenemases by the bacteria, mutation of porins, and efflux pump overexpression, which all decrease the drug concentration at the target site. Carbapenemases are categorized into different classes. Ambler molecular class A carbapenemases (KPC), class B Metallo-β-lactamases (MBLs) (VIM, IMP, NDM), and class D oxacillinases (OXA-48) are considered the most clinically prominent carbapenemases [2, 3]. So far, OXA-48, OXA-72, OXA – 23, OXA-51, OXA-172 and NDM1, NMD3, NDM4, NDM5, NDM7, NDM12 and NDM 13 are the commonly reported carbapenemases from Nepal [4, 5, 6, 7, 8]. The organism can have more than one type of carbapenemase-producing gene. Indian study reported CRKp from blood isolates (n = 115) producing blaNDM in 19% and blaOXA48-like in 13% and a combination of both in 28% (Veeraraghavan et al.) [9]. A similar study of 30 isolates of Carbapenem-resistant Acinetobacter baumannii reported that all 30 isolates (100%) were positive for blaOXA-51 like gene, blaIMP-1, and blaVIM-1 genes (Niranjan et al.) [10].

We are reporting a case series, which shows co-infection with IMP, VIM, NDM, KPC, and OXA-48, producing Escherichia coli and Klebsiella pneumoniae in patients admitted to ICU in a tertiary care center in Nepal. According to treatment guidelines, colistin, tigecycline, and fosfomycin are the drugs of choice for carbapenemase-producing organisms along with combining ceftazidime + avibactam or aztreonam + avibactam [11]. Amongst them, colistin and tigecycline are frequently used in Nepal, while other drugs are not available [4].
A 40-year-old female patient with diabetes presented to the emergency department with high fever, difficulty in urination, and burning micturition for four days. She was being treated with nitrofurantoin orally for three days and showed no improvement. On physical examination, she was febrile with renal angle tenderness. Her chest was clear. Her total leucocyte count (TLC) was 18000 cells/µl, with predominance of neutrophils. Her renal and liver functions were within normal limits. Urine microscopy showed 20–25 pus cells/ HPF. The patient was started piperacillin-tazobactam empirically after obtaining blood and urine cultures. The patient continued to have fever despite being on antibiotics. Antimicrobial therapy was changed to imipenem - cilastatin. Despite carbapenem therapy, she progressively worsened with a gradual drop in blood pressure, requiring inotopic support and admission to ICU.

E. coli was isolated from her blood and urine samples. The isolate was resistant to multiple drugs such as ceftriaxone, piperacillin, and tazobactam, cotrimoxazole, amikacin, levofloxacin, carbapenems (imipenem and meropenem), but was susceptible to colistin, tigecycline, and fosfomycin. She was administered colistin and tigecycline. Colistin was started with a loading dose of 9 million units (MU) followed by 3 (MU) three times a day. Tigecycline was given at a loading dose of 200 mg, followed by a maintenance dose of 100 mg bidaily. On the third day of treatment, she developed acute kidney (AKI) injury with an elevated serum creatinine level of 2.2 mg/dl. Treatment with colistin was stopped and Inj. Fosfomycin was started intravenously after procurement from India. 4 gm of fosfomycin TID was administered initially, and the drug was modified to 8 gm TID when serum creatinine normalized. The organism was tested for carbapenemase production and was found to be positive with the Modified Hodge test (Fig. 1). The isolates were further processed by Xpertcarba R, which showed NDM, KPC, and OXA-48 carbapenemase production (Fig. 2). After initiating treatment with intravenous fosfomycin, the patient became afebrile on the second day of therapy. Inotropes were tapered and stopped and AKI resolved. Fosfomycin was continued till the fourteenth day of the first negative blood culture. After completion of antimicrobial therapy, the patient was asymptomatic on follow-up after
CASE 2
A 52 years old alcoholic male presented with symptoms of fever, cough with expectoration for five days. A clinical diagnosis of community-acquired pneumonia was made, and he was prescribed Inj. Amoxicillin-clavulanic acid and oral azithromycin. There was no improvement with this empirical therapy, and hence the patient was shifted to a regional hospital where antibiotics were modified to piperacillin-tazobactam. Sputum and blood cultures were sent to aid in further clinical diagnosis. Radiological findings showed haziness in both right and left middle and lower zones. The patient had further deteriorated, and the antibiotic regimen was changed to intravenous meropenem after three days. He was referred to our tertiary care center in Kathmandu since he did not improve. On admission, he had severe respiratory distress with a worsening chest X-ray compared to graphy taken in the previous hospital. Sputum and blood cultures were reinvestigated. Intravenous and nebulized colistin, along with teicoplanin, was added to meropenem to cover for MRSA and MDR gram-negative organisms. His blood picture revealed leucocytosis with a TLC of 22030 cells/microliter. Liver function tests showed mild derangement, but his renal functions were normal. Subsequently, blood and sputum cultures yielded MDR Klebsiella pneumoniae. The strain was susceptible to tigecycline and colistin solely. Modified Hodge test (MHT) was performed and showed that the organism was an of carbapenemase producer. Xpert Carba R showed blaVIM, blaNDM, blaKPC, and blaOXA genes (Fig. 3). Treatment with intravenous colistin and tigecycline was continued for ten days. The patient was shifted to the ward on the fourth day of admission and later discharged with good clinical outcomes.

CASE 3
A 48-year-old male on antiviral therapy (tenofovir) for Hepatitis B was referred from a peripheral hospital in Nepal. His major complaints were high-grade fever and abdominal distension concordant with diffuse abdominal pain. Blood investigations showed neutrophilic predominance elevated TLC (18100 cells/microliter), with elevated liver enzymes ALT (95 U/L) and AST (88 U/L) and normal renal functions. Abdominal ultrasound reported ascites, and ascitic fluid analysis was performed with the suspicion of peritonitis reporting TLC 720cells/mm3 with 90% neutrophils and adenosine deaminase
(ADA) of 9 IU/L. Microscopy of the fluid showed gram-negative bacilli. The patient was given intravenous piperacillin and tazobactam in a peripheral hospital, but there was no clinical improvement. Hence, antibiotics were modified to Inj. Imipenem-cilastatin in a dose of 500 mg every six hours. A new onset hypotension developed, which was controlled with vasopressor drugs. An additional antimicrobial agent, colistin, was added in the ICU to cover resistant Gram-negative organisms when the culture reports were pending. E. coli was recovered in both the ascitic fluid and in the blood. The pathogen was susceptible to both colistin and tigecycline by the Kirby-Bauer disc diffusion method. This isolate showed blaIMP, blaVIM, blaNDM, and blaOXA genes (Fig. 4) by Xpert Carba R like the isolates in the previous cases, too. Based on these reports, treatment modality was modified from intravenous imipenem to tigecycline at a loading dose of 200 mg parenterally to 100 mg BD as a continuation therapy. The patient began to improve on the third day of therapy and was shifted to the ward on the fifth day.

**Discussion**

MDR Enterobacteriaceae are not routinely expected in community-acquired infections. Management of infections caused by these pathogens is challenging due to the limited availability of treatment options, both expensive and are associated with serious adverse effects.

K. pneumoniae carbapenemase (KPC) is the most common enzyme, causing carbapenem resistance, KPC-2 in particular. KPC-producing bacteria have spread all over the world, including Asia, but NDM-1 has the highest frequency of detection in Asia. The bacteria harboring these two genes are more dangerous and are resistant to carbapenem at a higher level than any other commonly encountered bacteria that have carbapenemase-encoding genes (including OXA-48, another popular carbapenemase produced by Enterobacteriaceae) [11, 12, 13, 14, 15]. Bloodstream infections caused by CRE have higher mortality (65.4%) compared to non-CRE infections (17.2%) (Li and Ye) [16]. Attributable death due to CRE is around 26-44% and hence the importance of early detection of carbapenem resistance in clinical isolate. Among the carbapenemases mortality caused by infection due to KPC-producing K. pneumoniae was between 40- 68% (Li and Ye) and up to 88% for NDM-1 producing Enterobacteriaceae. In recent studies, carbapenem minimum inhibitory concentrations
(MICs) for these two types of carbapenemase producers showed higher carbapenem MIC values [17]. The first report of the NDM-1 enzyme was available in 2009. It was detected in a Swedish patient who had traveled to India and was hospitalized in New Delhi in 2007. NDM-1 is a broad-spectrum beta-lactamase (carbapenemase) that can inactivate all beta-lactams except aztreonam. However, most NDM-1-positive strains also express the CMY-4 and CTX-M-15 beta-lactamases, which confer resistance to all beta-lactams. In a study conducted in Nepal, 13.6% of Acinetobacter baumannii isolates were carrying the blaNDM-1 gene [5, 13].

The presence of MBL genes on mobile genetic elements increases the chances of the presence of other drug resistance, causing genes. There may be a combination of carbapenemase genes (OXA 48, OXA 181, and VIM), aminoglycoside resistance genes (16s rRNA methylase), plasmid-associated cephalosporinase genes (CMY-16, CMY-58), class A genes (KPC), ESBL genes (TEM, CTX-M-15, SHV-12), macrolides resistance genes (esterase), qnr genes (qnrAB, qnr B1, qnr B2) and rifampin resistance gene. Thus, they confer resistance to multiple groups of antimicrobial agents [13, 14, 15]. An Indian study reported 5% community-acquired K. pneumoniae bloodstream infection as carbapenem-resistant (Veeraraghavan et al.)[9]. Our case of community-acquired pneumonia, along with bacteremia, also showed carbapenem resistance, which signifies the increasing carbapenem resistance in the community. This growing resistance may have significant implications on the empirical choice of antibiotics for community-acquired infection.

The problem of NDM-1 was not confined to hospital strains but was widespread in the community. These bacteria can colonize the gastrointestinal tract; hence fecal-oral transmission plays a significant role by transmitting infection through contaminated food, water, and hands [12]. Many phenotypic and genotypic (molecular) detection methods can identify carbapenem-resistant bacteria. These include automated systems, disc diffusion, MICs, selective agar, modified Hodge test, synergy tests (e.g., E-tests or double-disc tests), spectrometry, whole genome sequencing and molecular methods [2].

Currently, the treatment options for CRE infections remain very limited [1]. Two classes of antibiotics,
colistin, and tigecycline have shown in vitro activity against NDM-1- positive Enterobacteriaceae. An in vitro sensitivity analysis revealed that the 50% and 90% MICs for colistin were 0.5 μg/mL and 8 μg/mL, respectively, whereas those for tigecycline were 1 μg/mL and 4 μg/mL. Colistin is effective for such infections, but it has a toxic potential, nephrotoxicity in particular. In a few cases, fosfomycin and aminoglycosides are used. Carbapenems still play a major role in the treatment of CRE infections, particularly when used in the treatment of CRE with lower MICs as part of the combination therapy. These drugs are used either in higher doses, in combination with other active anti-CRE agents, or through double-carbapenem therapy (DCT) [17, 18].

In a study conducted by Zusman et al. in 2017, a comparison of polymyxin-based combination therapy with monotherapy reported a significant decrease in 30-day mortality at combination therapy arm compared to monotherapy [19]. The newer antibiotics, like CAZ/AVI, which inhibit KPC and OXA-48 but do not inhibit MBLs (NDM, VIM, and IMP), are not active against all CRE isolates [1]. In contrast, the monobactam antibiotics aztreonam (ATM), which is stable against MBLs but is hydrolyzed by many other β-lactamases (ESBL, Amp C, and cephalosporinases) are frequently co-produced by the MBL producing strains. Hence, the combination of CAZ/AVI and ATM has been proposed as a potential therapeutic regime against various infections caused by MBL-producing bacteria [1].

Table 1 - showing patient with organism, resistant genes, treatment and outcome

| Cases | Age, sex | Sample | Organism | Carbapenemase resistant genes | Treatment regimen dose and duration | Outcome |
|-------|----------|--------|----------|-----------------------------|-----------------------------------|---------|
| Case 1 | 40 years Femal e | Urine & blood | E. coli | blaNDM, blaKPC, blaOXA-48 | Fosfomycin for 14 days | improved |
| Case 2 | 52 years Male | Sputum & blood | Klebsiella pneumoniae | blaVIM, blaNDM, blaKPC, blaOXA-48 | Colistin & tigecycline for 10 days | improved |
| Case 3 | 48 years Male | Ascitic fluid & blood | E. coli | blaIMP, blaVIM, blaNDM, blaOXA-48 | Colistin & tigecycline for 10 days | improved |

Conclusion
Our case series show the presence of different types of carbapenemase production in the community-acquired bacteria isolated from none-hospitalized patients. These isolates all belong to the Enterobacteriaceae family with resistant genes like IMP, VIM, NDM, KPC, and OXA-48. Multiple resistance genes are present within one single isolate making it challenging to choose an adequate regimen. MDR organisms are present in both communities as well as in healthcare settings. This situation is alarming. Larger and multicentric studies should be carried out to disclose the exact burden of carbapenem resistance in the community. Physicians should be aware of increasing carbapenem resistance in clinical isolates and regulate their antibiotic use in clinical settings accordingly. Polymyxin, fosfomycin, tigecycline, minocycline, amikacin, ceftazidime-avibactam, and ceftazidime-avibactam-aztreonam are potential options for treating CRE but are limited by their side effect profile.

Declarations

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Declarations:

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Ethics approval

According to the local ethical guidelines, ethical approval is not required for a case series.

Consent to publish

For publication of this case series, written informed consent was taken from the patient.

Conflict of Interest statement

'Authors declare No conflicts of interest'
Authors’ contributions

RS established the diagnosis, reviewed the literature, and designed the manuscript. AJRM, NP, GSS, FF, SS, MS, AAR, MK, SS, MA, RJ, SK and HE reviewed the literature and prepared the article. All authors read and approved the final version of the manuscript.

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Figures
Figure 1

Modified Hodge test showing indentation due to Carbapenemase production by test organism.
Figure 2

E. coli showing positive of NDM, KPC and OXA-48 carbapenemase by detecting blaNDM, blaKPC and blaOXA-48 genes on Xpert Carba R.
Figure 3

K. pneumoniae showing positive of VIM, NDM, KPC and OXA-48 carbapenemase by detecting blaVIM, blaNDM, blaKPC and blaOXA-48 genes on Xpert Carba R.
Figure 4

E. coli showing positive of IMP, VIM, NDM and OXA-48 carbapenemase by detecting blaIMP, blaVIM, blaNDM and blaOXA-48 genes on Xpert Carba R.