Molecular characterization of a multidrug-resistant/pandrug-resistant nosocomial polymicrobial infection with *Klebsiella pneumoniae*, *Providencia rettgeri*, and *Acinetobacter baumannii* from Rural Maharashtra, India

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The emergence of resistance against commonly used antibiotics has become a serious global concern. The rapid development of antibiotic resistance exhibited by *Enterobacteriaceae* has caused an increasing concern regarding untreatable bacterial infections. Here, we isolated four pathogens from a geriatric female patient who was hospitalized for a month with ventilator-associated pneumonia (VAP) and fever. The organisms isolated from the tracheal aspirates and urine included *Klebsiella pneumoniae*, pandrug-resistant *Providencia rettgeri*, and *Acinetobacter baumannii*. Resistome analysis indicated that the bacterial isolates from the polymicrobial infection were multiple-drug resistant and pandrug resistant clones. Molecular characterization revealed presence of *bla*EM₁ in *K. pneumoniae*, *P. rettgeri* and *A. baumannii*. The *bla*TEM₁ and *bla*NDM₁ genes were present in *P. rettgeri* and *A. baumannii* whereas the *bla*TEM₁V, *bla*NDM₁ and *bla*OXA₂₃ traits were present in *A. baumannii* isolates. The patient has died due to the unavailability of effective antimicrobial treatment for this drug-resistant polymicrobial infection.

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INTRODUCTION

Microorganisms that are primarily involved in antibiotic resistance are called the “ESKAPE” pathogens, and include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species*, capable of “escaping” from commonly used antibacterial treatments (Boucher et al., 2009). *A. baumannii* has emerged as a highly challenging pathogen due to its specific antibiotic resistance characteristics (Boucher et al., 2009). Moreover, reports of extensively drug-resistant and pandrug-resistant *K. pneumoniae* (XDR-KP and PDR-KP) cases are increasing worldwide (Fiorellakrap et al., 2018). *K. pneumoniae* is the most clinically relevant *Klebsiella* species and is responsible for >70% of infections (Hansen et al., 1998). Antimicrobial resistance has become a global crisis because of escalating resistance coupled with diminished antibiotics in the developmental pipeline. A recent report estimates that by 2050, antimicrobial resistance-related mortality will be 10000000/year (de Kraker et al., 2016).

The rapid emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) worldwide has led to the concern that these infections may be soon untreatable. Management of infections caused by *K. pneumoniae* has been complicated by antimicrobial resistance, especially that against carbapenems. Whole genome sequence analyses of six extensively drug resistant (XDR) enteric pathogens isolated at New Delhi revealed multiple mobile genetic elements that were physically linked to antibiotic resistance traits. Thus, these elements seem to be responsible for disseminating drug resistance among organisms through underlying mechanisms of horizontal gene transfer and resistance to commonly used antibiotics (Kumar et al., 2017). Resistance to carbapenems in *K. pneumoniae* involves multiple mechanisms, including production of carbapenemases, such as KPC, NDM, VIM, and OXA-48-like (Johann et al., 2015).

A 10-year study at Nashik, India (Odsbu et al., 2018, Lokhande et al., 2019), revealed a significantly higher proportion of non-susceptible and extended-spectrum β-lactamase (ESBL)-producing isolates from inpatients than those from outpatients for both, *Escherichia coli* and *Klebsiella* spp. A higher proportion of non-susceptible isolates indicates a great need to focus on the optimal use of antibiotics to reduce the development of antibiotic resistance.

Diverse risk factors associated with multidrug-resistance (MDR) in *A. baumannii* and other *Enterobacteriaceae* members suggest that a separate outbreak investigation should be performed in each hospital setting. Development of innovative control strategies is needed to limit the spread of these pathogens (Falagas & Kopterides, 2006).

In this study, we aimed to elucidate the mechanisms underlying drug resistance exhibited by prevalent pathogens responsible for unresponsiveness to the treatment administered to the patient. *K. pneumoniae*, *P. rettgeri*, and *A. baumannii* were isolated from the urine and tracheal aspirate of the patient on admission to the Somani Hospital, Barshi, Maharashtra, India.

CASE PRESENTATION

A 64-year-old female patient was hospitalized in Barshi with altered behavior, history of fall, and intracranial hemorrhage; the patient was put on a ventilator. Earlier, for 4 weeks, she received treatment at the Neurology Centre in Solapur, Maharashtra, and upon stabiliza-
tion, she was moved to the Dr. Yogesh Somani Hospital, Barshi. During treatment, the patient developed a ventilator-associated pneumonia (VAP) and was administered piperacillin/tazobactam, meropenem, tigecycline, and colistin without a culture and susceptibility testing (Fig. 1, first hospitalization).

On arrival at the Somani Hospital, the patient had a fever, was drowsy and arousable to the delayed premonition syndrome (DPS). She did not respond to verbal command, was on tracheostomy and discontinued from the ventilator use. She was treated with oral fluconazole and intravenous imipenem. Tigecycline was included in the treatment regimen without prior confirmation obtained by using an antibiogram or susceptibility testing. This therapy continued until the 16th day of hospitalization.

The patient was found to have bacteriuria and dead pus cells in the urine; therefore, urine culture and a susceptibility test was performed on the 32nd day of hospitalization; a mixed infection of Candida albicans and Proteus rettgeri was found. Based on susceptibility analysis, fluconazole, meropenem, and moxifloxacin was administered for the next 8 days. The fever continued and tigecycline was administered again for 7 days. On day 32, tracheal aspirate were tested for culture; K. pneumoniae and A. baumannii KSK0 were identified and isolated.

Based on the culture results and susceptibility analysis, treatment with meropenem and moxifloxacin was started. On day 47, she had high-grade fever, deteriorated CNS status, and was put on a ventilator support; the treatment was augmented and gentamicin was initiated in addition to meropenem and moxifloxacin.

On day 48, the culture and susceptibility analysis for the tracheal aspirate revealed A. baumannii KSK1, and gentamicin injections were administered along with the treatment on day 53, until the death of the patient on day 66. The cause of death was poly-microbial infection caused by resistant pathogens. Available antibiotics and treatment were insufficient.

MATERIALS AND METHODS

The isolates were collected during December 2017 at Barshi town in Maharashtra, India. Isolates were cultured on blood and MacConkey agars for purification. Well isolated, similar looking colonies were sub-cultured on tryptase soy agar and preserved in glycerol at -70°C for further analysis. Isolates were identified by the VITEK-2 (bioMérieux) system.

Susceptibility testing. The four selected isolates: K. pneumoniae, A. baumannii KSK0, and A. baumannii KSK1 from tracheal aspirates, and P. rettgeri from urine, were susceptibility tested using the VITEK-2 (bioMérieux) system. The panel covered a broad range of antibiotics to estimate resistance and guide the antibiotic therapy. The isolates were susceptibility tested by also using a reference broth microdilution minimum inhibitory concentrations (MIC) determination method, as described by the CLSI (M07, A10).

Briefly, a serial two fold dilutions of the antibacterial agents were made in cation-adjusted Muller-Hinton broth (BD, USA) in the presence or absence of a fixed inhibitor concentration. Bacterial suspensions of 0.5 McFarland turbid-

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Figure 1. A 64 year old female patient was hospitalized twice at two different hospital. Upon first admission, which was due to neurological complications, she was treated empirically for a month with Piperacillin/Tazobactam, Meropenem, Tigecyclin (a week) and Colistin. During the second hospitalization, culturing was performed for the tracheal fluid and one time culture was done from urine. Patient was found to have infected with yeast and three types of MDR, XDR and PDR bacterial pathogens.
ity equivalents were prepared in sterile 0.85% Saline (NaCl) and were appropriately diluted to obtain a final cell density of 2–8×10^8 CFU/mL in the antibiotic containing medium. The plates were incubated for 18 h at 37°C. MICs were recorded as the lowest antibiotic concentration showing no visible growth of an organism. Categorical interpretations of all comparator agents were those found in the CLSI breakpoint tables (M100, S26). Quality control was performed using *Escherichia coli* ATCC 25922. All quality control MIC results were within acceptable ranges published in CLSI documents. The antibiotic panel included ceftazidime in combination with avibactam to detect the presence of KPC, Ambler Class C, and OXA-48 enzymes while, meropenem-EDTA combination exhibited an MBL enzyme.

**Genotype Determination.** All of the isolates were tested for the presence of *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, and *bla*<sub>OXA</sub> genes by a PCR assay using specific primers (Table 1). The bacterial lysate was used as template DNA with a final reaction volume of 25 μl containing 10× buffer, 2.5 mM of dNTPs, 15 mM MgCl<sub>2</sub>, 100 pM of each oligonucleotide primer, 1 U of Taq polymerase and 2 μl of bacterial lysate. PCR was carried out in a thermal cycler using specific annealing temperatures as shown in Table 1. Amplified products were resolved in an agarose gel. The band size for the specific amplified genes was compared with the control samples in the same run.

| β-lactamase gene | Primer Sequence | Amplicon Size (bp) | Annealing temp (°C) | Annealing location | Reference |
|------------------|-----------------|--------------------|---------------------|-------------------|-----------|
| *bla*<sub>CTX-M</sub> group I | GACGATGTCGCTGGTGGAC | 499 | 55 | 416–435 | NA |
| &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&n...
intermediate resistance to gentamicin and tigecycline, and susceptibility to colistin (Table 2).

**Resistotype and Genotyping studies**

The VITEK-2 and broth microdilution MIC susceptibility results shown in Table 3 were validated by performing PCR amplifications for various β-lactamase traits. Results presented in Fig. 2 (a, b, c) and summarized in Table 4, show that the **bla**\(_{\text{TEM-1}}\) variant was present in all four clinical isolates, whereas **bla**\(_{\text{NDM-1}}\) was present only in *P. rettgeri*. Presence of **bla**\(_{\text{NDM-1}}\) explains the susceptibility of *P. rettgeri* to meropenem in combination with the metal ion chelating agent EDTA. Interestingly, **bla**\(_{\text{NDM-1}}\) was found in *A. baumannii* KSK0 and *A. baumannii* KSK1, along with **bla**\(_{\text{NDM-1}}\) isolated at different stages. All clinical isolates were genetically ESBL positive, however, *A. baumannii* KSK0 and *A. baumannii* KSK1 had three β-lactamases, indicating evolution of a complicated drug resistance mechanism. Virulent and resistant *K. pneumoniae* strains are a significant cause of hospital-acquired infections. Studies performed in Iran reported a high prevalence of resistance against several antibiotics, and the simultaneous presence

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### Table 2. Antibiotic susceptibility of pathogens isolated from the tracheal secretion and urine by VITEK-2

| Antimicrobial agent | Symbol | **Klebsiella pneumoniae** | **Providencia rettgeri** | **Acinetobacter baumannii**<br> KSK0 | **Acinetobacter baumannii**<br> KSK1 |
|---------------------|--------|-------------------------|--------------------------|-----------------------------------|-----------------------------------|
|                     |        | MIC                     | R/S                      | MIC                               | R/S                               |
| **Amikacin**        | AK     | ≤2                      | S                        | ≥64                               | R                                 |
| **Aztreonam**       | AT     | ≥32                     | R                        | ≥64                               | R                                 |
| **Cefepime**        | CPM    | ≥1                      | S                        | ≥32                               | R                                 |
| **Cefoperazone-Sulbactam** | C-S   | ≥32                     | R                        | ≥64                               | R                                 |
| **Ceftazidime**     | CAZ    | ≥64                     | R                        | ≥64                               | R                                 |
| **Ceftiraxone**     | CTR    | ≥64                     | R                        | ≥64                               | R                                 |
| **Cefuroxime**      | CXM    | ≥64                     | R                        | ≥64                               | R                                 |
| **Ciprofloxacin**   | CIP    | ≤0.25                   | S                        | ≥4                                | R                                 |
| **Colistin**        | CL     | ≤0.5                    | S                        | ≥2                                | R                                 |
| **Doxycycline**     | DO     | *                       | *                        | R                                 | R                                 |
| **Ertapenem**       | ETP    | ≤0.5                    | S                        | ≥8                                | R                                 |
| **Gentamicin**      | GEN    | ≤1                      | S                        | ≥16                               | R                                 |
| **Imipenem**        | IPM    | ≤0.25                   | S                        | ≥16                               | R                                 |
| **Levofloxacin**    | LE     | ≥8                      | R                        | ≥16                               | R                                 |
| **Meropenem**       | MRP    | ≤0.25                   | S                        | ≥16                               | R                                 |
| **Minocycline**     | MI     | *                       | *                        | R                                 | ≤1 S                              |
| **Moxifloxacin**    | MO     | ≥2                      | R                        | ≥16                               | R                                 |
| **Piperacillin-Tazabactam** | PIT    | ≥128                    | R                        | ≥128                              | R                                 |
| **Tigecycline**     | TIG    | 4.00                    | R                        | 4.00                              | I                                 |
| **Trimethoprim-Sulphomethoxazole** | COT | ≤20                     | S                        | ≥320                              | R                                 |

MIC: minimum inhibitory concentration expressed in mg/L, S: susceptible, R: resistant, I: Intermediate. Interpretation of the drugs marked with (*) has been deduced based on phenotype of the isolate by VITEK-2. Hence MIC value cannot be reported.

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Figure 2. Amplification of **OXA** β-lactamase trait (a), **NDM** β-lactamase trait (b), and **TEM-1** β-lactamase trait (c) among tracheal secretion and urine isolated from a patient with a polymicrobial infection.

Lane 1: DNA ladder, Land 2: *K. pneumoniae*, Lane 3: *P. rettgeri*, Lane 4: *A. baumannii* KSK0 and Lane 5: *A. baumannii* KSK1
of certain virulence factors and MDR genes, contributing to a crucial public health issue (Ranjbar et al., 2019). Carbapenemases and ESBLs are responsible for pandrug-resistant (PDR) in K. pneumoniae clinical samples in Maharashtra, India (Lokhande et al., 2019). In their study, ESBL resistance was observed in 310 (88.57%) isolates and carbapenemase in 181 (51.71%) isolates; these were the primary mechanisms underlying antibiotic resistance. A total of 29 (8.28%) K. pneumoniae PDR isolates and 52 (14.85%) isolates susceptible to colistin alone were found. They found extreme drug resistance in 135 (38.57%) of the K. pneumoniae isolates.

The emergence and spread of MDR, ESBL producing carbapenem-resistant members of Enterobacteriaceae has become a worldwide health problem. A study in Bangkok, Thailand, revealed a unique prevalence of carbapenemase genes, where bla*DM and bla*OXA-23 were predominant (Laolerd et al., 2018).

Providencia species are intrinsically resistant to colistin and tigecycline, making the treatment of MDR Providencia spp. challenging. Carbapenem-resistant Providencia spp. have been reported (Abdallah & Balshi, 2018). An outbreak of carbapenem-resistant P. rettgeri, involving 4 patients admitted to intensive care and high-care units at a tertiary hospital was reported. Their clinical and demographic characteristics were studied; experiments revealed that all P. rettgeri strains were resistant to carbapenems - imipenem, ertapenem, and meropenem (Tshisevhe et al., 2016). Our results are in line with these referred studies that the P. rettgeri strain isolated from urine of our patient was a PDR strain.

The MDR A. baumannii has been recognized as clinically significant. Numerous reports relay the spread of A. baumannii in hospital settings, leading to nosocomial outbreaks with increased mortality. However, many Acinetobacter spp. can also cause nosocomial infections. A review focused on the role of Acinetobacter spp. as nosocomial pathogens along with their persistence, antimicrobial resistance patterns, and epidemiology has been recently published (Almasaudi, 2018).

Antimicrobial resistance among Acinetobacter spp is a global threat (Clark et al., 2016). A. baumannii is a major cause of healthcare-associated infections. MDR A. baumannii is a rapidly emerging pathogen, especially in the intensive care units, causing infections including bacteremia, pneumonia/VAP, meningitis, urinary tract infection, central venous catheter-related infection, and wound infection. An optimal treatment for A. baumannii nosocomial infections has not been established (Clark et al., 2016). However, the antibiotics that are usually effective against A. baumannii infections include carbapenems, polymyxins E and B, sulbactam, piperacillin/tazobactam, tigecycline, and aminoglycosides. Carbapenems (imipenem, meropenem, doripenem) are the mainstay of A. baumannii treatment; however, carbapenem-resistant Acinetobacter strains have been recently reported. These bacteria commonly present resistance to multiple antimicrobial agents, including carbapenems and polymyxins; hence, they are considered the paradigm of MDR or PDR bacteria. The XDR A. baumannii KSK0 and A. baumannii KSK1 isolates were difficult to treat as their MIC values were much higher than the prescribed doses for these antibiotics.

The indiscriminate and widespread antibiotic use causes rise of the resistant A. baumannii strains. A study performed in Chennai, India, reported the frequency of MDR (71.23%) and XDR (39.72%) for A. baumannii iso-

### Table 3. MIC established by broth double dilution method for the four clinical isolates

| Antimicrobial Agents | Klebsiella pneumoniae | Acinetobacter baumannii KSK0 | Providencia rettgeri | Acinetobacter baumannii KSK1 |
|----------------------|-----------------------|-----------------------------|---------------------|-----------------------------|
| Ceftriaxone          | 0.12                  | >64                         | >64                 | >64                         |
| Ceftriaxone+Clavulanic acid | 0.12              | >64                         | >64                 | >64                         |
| Ceftriaxone+avibactam | 0.06                 | >64                         | >64                 | >64                         |
| Cefepime             | <0.03                 | >64                         | >64                 | >64                         |
| Pipercillin-Tazobactam | >128            | >64                         | >64                 | >64                         |
| Meropenem            | <0.03                 | >64                         | >64                 | >64                         |
| Meropenem+EDTA       | <0.03                 | 64                          | 0.25                | 64                          |
| Imipenem             | 0.25                  | >64                         | >64                 | >64                         |
| Colistin             | 0.12                  | 1                           | >32                 | 1                           |
| Tigecycline          | 1                     | 2                           | 2                   | 2                           |
| Meropenem            | 0.06                  | 16                          | >64                 | 16                          |
| Levofloxacin         | 2                     | >256                        | >256                | >256                        |
| Trimethoprim-sulfamethoxazole | 2                  | >64                         | >64                 | >64                         |

MIC, minimum inhibitory concentration; ceftazidime with fixed clavulanic acid 4 mg/L, ceftazidime with fixed avibactam 4 mg/L, piperacillin/tazobactam 4 mg/L, EDTA, ethylenediaminetetraacetic acid at fixed 200 mg/L.

### Table 4. Phenotypic and genotypic resistance mechanism for the four clinical isolates

| Organism      | Phenotype | Genotype                |
|---------------|-----------|-------------------------|
| K. pneumoniae | WT        | bla*DM                  |
| A. baumannii  | KSK0      | bla*DM, bla*OXA-23      |
| A. baumannii  | KSK1      | bla*DM, bla*OXA-23      |
| P. rettgeri   | Class B   | bla*DM                  |
| A. baumannii  | KSK0      | bla*DM, bla*OXA-23      |
| A. baumannii  | KSK1      | bla*DM, bla*OXA-23      |
| WT, wild type |           |                         |

of certain virulence factors and MDR genes, contributing to a crucial public health issue (Ranjbar et al., 2019). Carbapenemases and ESBLs are responsible for pandrug-resistance (PDR) in K. pneumoniae clinical samples in Maharashtra, India (Lokhande et al., 2019). In their study, ESBL resistance was observed in 310 (88.57%) isolates and carbapenemase in 181 (51.71%) isolates; these were the primary mechanisms underlying antibiotic resistance. A total of 29 (8.28%) K. pneumoniae PDR isolates and 52 (14.85%) isolates susceptible to colistin alone were found. They found extreme drug resistance in 135 (38.57%) of the K. pneumoniae isolates.

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The indiscriminate and widespread antibiotic use causes rise of the resistant A. baumannii strains. A study performed in Chennai, India, reported the frequency of MDR (71.23%) and XDR (39.72%) for A. baumannii iso-
lates (Girija & Priyadharsini, 2019). That study stated that periodical antibiotic surveillance is essential to curb the emergence of MDR and XDR A. baumannii in hospital environments, improving patient care using alternate drugs of choice or a combination therapy. A study in Algeria highlighted the high prevalence of imipenem-resistant A. baumannii in the Algiers hospitals, mediated by blaOXA-23-like, blaOXA-24-like, and blaOXA-35, genes (Khorisi et al., 2015). A study performed in Turkey revealed that the prominent genes responsible for carbapenem resistance in clinical A. baumannii strains were blaOXA-51 and blaOXA-23, and the high prevalence of clones may constitute a threat for hospitalized patients (Direkli et al., 2016).

In general, 95% of cases in rural India are treated empirically without culture and antibiogram reports. In spite of culturing facilities, treatment for a long duration and a polymicrobial infection can result in poor prognosis and outcomes. Similarly, the presented case study demonstrates the acquisition of nosocomial pathogens while undergoing treatment for neurological complications. The presence of MDR/XDR/PDR pathogen clones in the hospital environment makes the critical surgeries complicated, and the pathogens' elevated MICs add more difficulty to find a successful therapy.

CONCLUSION

A female geriatric patient was found to suffer a polymicrobial infection caused by K. pneumoniae, P. rettgeri, A. baumannii, and Candida albicans. Co-existence of the ESBL traits: blaTEM-1, blaTEM-3, blaOXA-23, caused high MIC values posing difficulty to meet a desired dose of antibiotics and in turn led to failure.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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REFERENCES

Abdallah M, Balshi A (2018) First literature review of carbapenem-resistant Providencia and New Members New Infect 25: 16–23. PMID: 29983980.

Almasaud IB (2018) Acinetobacter spp. as nosocomial pathogens: Epidemiology and resistance features. Saudi J Biol Sci 25: 586–596. https://doi.org/10.1016/j.sjbs.2016.02.009

Michael Scheld, Brad Spellberg, John Bartlett Boucher HW, Talbot GH, Bradley JS, Edwards JR, Gellert D, Riez LB, Scheld M, Spellberg B, Bartlett J (2009) Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 48:1–12. https://doi.org/10.1086/595011

Candem ED, Akoz N (2015) Klebsiella pneumoniae: characteristics of carbapenem resistance and virulence factors. Acta Biochim Pol 62: 867–874. https://doi.org/10.18388/abp.2015._1148

Clark NM, Zhan GD, Lynch JP 3rd (2016) Emergence of antimicrobial resistance among Acinetobacter species: a global threat. Curr Opin Crit Care 22: 491–499. https://doi.org/10.1097/MCC.0000000000000337.

CLSI (2012) Performance Standards for Antimicrobial Susceptibility Testing: Approved standard M100-S22, 22nd Informational Supplement Wayne, PA: Clinical and Laboratory Standards Institute

Dallenne C, Da Costa A, Decre D, Favier C, Arlet G (2010) Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J Antimicrob Chemother 65: 490–495. https://doi.org/10.1093/jac/dkp498

De Kraker ME, Stewardson AJ, Harbarth S (2016) Will 10 million people die a year due to antimicrobial resistance by 2050? PLoS Med 13: e1002184. https://doi.org/10.1371/journal.pmed.1002184.

Direkli S, Copard Cicak A, Kargoz A, Aydogan Ejder N, Oktyay F, De-dialogha N, Orgamus OB, Durmus R (2016) Antimicrobial evaluation susceptibility and molecular characterization of multidrug-resistant Acinetobacter baumannii isolated in an university hospital. Microb Res 169: 522–534. PMID: 27124957.

Falagas ME, Kopterides I (2010) Risk factors for the isolation of multidrug-resistant Acinetobacter baumannii and Pseudomonas aeruginosa: a systematic review of the literature. J Hosp Infect 64: 7–15. https://doi.org/10.1016/j.jhin.2006.04.015

Girija AS, Priyadharsini JV (2019) CLSI based antibiogram profile and the detection of MDR and XDR strains of Acinetobacter baumannii isolated from urine samples. Med J Islam Repub Iran 8: 33:3. https://doi.org/10.34171/mjiri.33.33.

Hansens DE, Gottschau A, Kolmos HJ (1998) Epidemiology of Klebsiella bacteraemia: a case control study using Escherichia coli bacteraemia as control. J Hosp Infect 38: 119–132. PMID: 9522290.

Lokhande SR, Pawar ST, Karad DD (2019) Extended and Pan-drug resistance in Klebsiella pneumoniae due to carbapenemase and extended spectrum beta-lactamases. J Med Sci Clin Res 7: 498–509. https://doi.org/10.18535/jmscr/v7i12.91.

Khorisi K, Messay Y, Hamidi M, Ammari H, Bakour R (2015) High prevalence of multidrug-resistance in Acinetobacter baumannii and dissemination of carbapenemase-encoding genes blaOXA-23-like, blaOXA-24-like and blaNDM-1 in Algiers hospitals. Asian Pac J Trop Med 8: 438–446. https://doi.org/10.1016/j.aptm.2015.05.001.

Krapp F, Ozer EA, Qi C, Hauser AR (2018) Case report of an extensively drug resistant Klebsiella pneumoniae infection with genomic characterization of the strain and review of similar cases in the United States. Open Forum of Infectious Diseases 5: ofy074. https://doi.org/10.1093/ofid/ofy074.

Kumar P, Bag S, Ghosh TS, Dev P, Dayal M, Saha B, Verma J, Pant A, Saxena S, Desigamani A, Rana P, Kumar D, Sharma NC, Han-duce P, Maiti TK, Mukhopadhyay AK, Bhadra RK, Nair B, Ram-murthy T, Das B (2017) Molecular insights into antimicrobial resistance axes of multidrug-resistant enteric pathogens isolated from India. Sci Rep 7: 14468. https://doi.org/10.1038/s41598-017-14791-1.

Laerdel W, Akeda Y, Preepreeyaroj I, Ratthawongjaratik P, Santanirand P (2018) Carbapenemase-producing carbapenem-resistant Enterobacteriaceae from Bangkok, Thailand, and their detection by the carbapenemase gene (blaOXA and blaNDM) analysis. J Med Sci Clin Res 7: 498–509. https://doi.org/10.18535/jmscr/v7i12.91.

Mlynarek P, Roderova M, Kolar M (2016) Primer evaluation for PCR and its application for detection of carbapenemases in Enterobacteriaceae. J Med Sci Clin Res 7: 498-509. https://doi.org/10.18535/jmscr/v7i12.91.

Pitout JD, Nordmann P, Poirel L (2015) Carbapenemase-producing Klebsiella pneumoniae, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother 59: 5873–5884. https://doi.org/10.1128/AAC.00191-15.

Poirel L, Dortet L, Bernabé S, Normann P (2011) Genetic features of blaNDM-1-positive Enterobacteriaceae. Antimicrob Agents Chemother 55: 5403–5407. https://doi.org/10.1128/AAC.00576-07.

Ranbar R, Fatamah Kelishadrokhi A, Chahelgerdi M (2019) Molecular characterization, serotype, and phenotypic and molecular evaluation of antibiotic resistance of the Klebsiella pneumoniae strains isolated from different types of hospital-acquired infections. Infect Drug Resist 12: 605–611. https://doi.org/10.2147/IDR.S198369.

Tsilivesve VS, Lekalakala MR, Tshuma N, Janse van Rensburg S, Mhlele N (2016) Outbreak of carbapenem-resistant Providencia rettgeri in a tertiary hospital. S Afr Med J 107: 31–33.

Woodford N, Elliotting JM, Gough J, Towner JK, Ward ME, Brown S, Ames SG, Livermore DM (2006) Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents 27: 351–353.