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Emergence of nosocomial-acquired extensively drug-resistant and pandrug-resistant Enterobacterales in a teaching hospital in Kuwait

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Abstract:

Background: The emergence and high ascendancy of infections caused by extensively-drug-resistant (XDR) and pandrug-resistant (PDR) Enterobacterales isolates is a serious clinical and public health challenge. Isolation of PDR Gram-negative bacteria (GNB) in clinical setting is very rare and rarer is the infection caused by XDR GNB. Apart from restricted therapeutic options, these infections are associated with increased mortality and morbidity. Urgent studies to re-evaluate existing therapeutic options and research into new antibiotic molecules are desperately needed. The objectives of this study are to report the emergence of rarely encountered multidrug-resistant (MDR), difficult-to-threat, CRE infections in our hospital and investigate their molecular epidemiology.

Methodology: This was a retrospective observational analysis of six patients with severe infections caused by XDR and PDR Enterobacterales isolates at Mubarak AL Kabeer Teaching Hospital, Jabriya, Kuwait, over a period of one and half years. The mechanisms of resistance in these isolates were then prospectively investigated by molecular characterization and genomic studies.

Results: The majority of infections were caused by *Klebsiella pneumoniae* (83.3%, 5/6) and one (16.6%) was caused by *Escherichia coli*. Three patients had bloodstream infection (BSI), one had both BSI and urinary tract infection (UTI), one had respiratory tract infection, and the last one had UTI. Two patients were infected with OXA-48 producers, one patient was infected with NDM-1 producer, one patient was infected with NDM-5 producer, one patient was infected with both NDM-1 and OXA-48 producer and the last patient was infected with both NDM-5 and OXA-181 producer. For definite treatment, all patients received combination therapy. The mortality rate was high (50.0%).

Conclusion: The high mortality rate associated with XDR and PDR Enterobacterales infections and the limited antimicrobial options for treatment highlight the need for improved detection of these infections, identification of effective preventive measures, and development of novel agents with reliable clinical efficacy against them.

Keywords: Extensively-resistant; pandrug-resistant; Enterobacterales; nosocomial; infections; treatment; resistance genes; Kuwait

Émergence d'Entérobactéries nosocomiales acquises ultrarésistantes aux médicaments et pandrug-résistantes dans un hôpital universitaire au Koweït

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Résumé:

Contexte: L'émergence et la montée en puissance des infections causées par des isolats d'entérobactéries ultrarésistantes (XDR) et pandrug-résistantes (PDR) constituent un sérieux défi clinique et de santé publique. L'isolement de bactéries Gram-négatives PDR (GNB) en milieu clinique est très rare et plus rare est l'infection causée par XDR GNB. En dehors des options thérapeutiques restreintes, ces infections sont associées à une augmentation de la mortalité et de la morbidité. Des études urgentes pour réévaluer les options thérapeutiques existantes et la recherche de nouvelles molécules antibiotiques sont désespérément nécessaires. Les objectifs de cette étude étaient de signaler l'émergence d'infections à CRE multirésistantes (MDR), difficiles à menacer, rarement rencontrées dans notre hôpital et d'enquêter sur leur épidémiologie moléculaire.

Méthodologie: Il s'agissait d'une analyse observationnelle rétrospective de six patients atteints d'infections graves causées par des isolats d'entérobactéries XDR et PDR à l'hôpital universitaire Mubarak AL Kabeer, Jabriya, Koweït, sur une période d'un an et demi. Les mécanismes de résistance de ces isolats ont ensuite été étudiés de manière prospective par caractérisation moléculaire et études génomiques.

Résultats: La majorité des infections ont été causées par *Klebsiella pneumoniae* (83,3%, 5/6) et une (16,6%) a été causée par *Escherichia coli*. Trois patients avaient une infection du sang (BSI), un avait à la fois une BSI et une infection des voies urinaires (UTI), un avait une infection des voies respiratoires et le dernier avait une UTI. Deux patients ont été infectés par des producteurs d'OXA-48, un patient a été infecté par un producteur de NDM-1, un patient a été infecté par un producteur de NDM-5, un patient a été infecté par un producteur de NDM-1 et d'OXA-48 et le dernier patient a été infecté avec le producteur NDM-5 et OXA-181. Pour un traitement définitif, tous les patients ont reçu une thérapie combinée. Le taux de mortalité était élevé (50.0%).

Conclusion: Le taux de mortalité élevé associé aux infections XDR et PDR Enterobacterales et les options antimicrobiennes limitées pour le traitement soulignent la nécessité d'améliorer la détection de ces infections, l'identification de mesures préventives efficaces et le développement de nouveaux agents avec une efficacité clinique fiable contre elles.

Mots-clés: extrêmement résistant; résistant aux pandrogues; les Entérobactéries; nosocomiale; infections; traitement; gènes de résistance; Koweit

Introduction:

Global emergence of multidrug-resistant (MDR) Gram-negative bacteria is of major public health concern because of limited treatment options, increased morbidity and mortality, and lack of uniform infection prevention and control guidelines (1,2). Mortality rates reported with carbapenemase-producing *Klebsiella pneumoniae* infections vary from 22-72% (2-5). This wide range in mortality rates is dependent on the types of population studied and analyzed such as age, underlying disease and comorbidity. The rate may also be affected by inclusion of patients colonized by multi-drug resistant organisms (MDROs) rather than true infections with MDROs, which is what can affect the patient outcome and success of therapy.

Carbapenems are a class of antibiotics considered the drugs of choice for treatment of life-threatening infections (6). The emergence of carbapenem-resistant Enterobacterales (CRE) has added a new dimension to the burden of limited therapeutic options available to clinicians. In addition, these MDROs have the propensity to spread rapidly as attested to by their worldwide spread in India, Pakistan, United Kingdom, the Gulf region and in some low-income resource countries like Nigeria (7-9).

The Centers for Disease Control and Prevention (CDC) has noticed an increase in CRE-related infections in the US and consequently advised on possible procedures to avoid their spread (10). As previously seen with ESBLs, patients colonized with CRE are at higher risk of infections due to these CREs (11).

In recent years, cases of extensively drug-resistant (XDR) and pandrug-resistant (PDR) Enterobacterales infections with unfavorable consequences have been reported around the globe (2,12-14). The choice of appropriate antimicrobial agents is a big challenge due to their XDR or PDR phenotype and co-resistance with other β-lactams, aminoglycosides, quinolones, and fosfomycin. Pandrug-resistant Enterobacterales have been reported sporadically in many countries (14-17). However, PDR Enterobacterales associated with clinical infections in Kuwait are still rare and have only been reported in a few studies (17).

In this communication, we report the characteristic features of patients with infections caused by XDR and PDR Enterobacterales isolates, the molecular analysis of the genes mediating their resistance phenotypes and the associated relatively high mortality rates seen in Kuwait.

Patients and methods:

Study setting:

This study was conducted on infected patients seen and managed at the Mubarak AL Kabeer Teaching Hospital, Jabriya, Kuwait.

Study design:

The first part of the study was a retrospective observational analysis of six patients with severe infections caused by XDR and PDR Enterobacterales isolates. The isolates were then secondarily investigated prospectively by molecular characterization and genomic analyses.

Ethical approval:

Institutional ethical approval was obtained from the Health Sciences Centre Ethical Committee, Health Sciences Centre, Kuwait University (permit number VDR/EC/4025). No waiver was obtained for the study. Collection of the specimens was conducted according to the Declaration of Helsinki and with particular institutional ethical and professional standards. No additional specimens were collected from the patients for this study. Informed consent was obtained from all participants of the study or the legally authorized representative for unconscious patient. The patient identities were kept anonymous.

Data collection:

The data concerning the patients were collected retrospectively from laboratory and medical in-patient records during a period of one and half year (January 2018 – June 2019). Important bio-data and useful information collected were age, gender, ethnic origin, past medical history prior to admission, diagnosis on admission, date infected, length of hospital stays, history of previous hospital admission, previous antibiotics used, co-morbidities, travel history outside the country within 12 months preceding admission and countries visited.

Case presentations:

Case 1:

This was a 39-year-old lady admitted with a slipped right-sided Double-J (DJ) stent and symptoms of urinary tract infection (UTI) on April 11, 2019. Her past medical history included prior admission for right kidney obstruction, hydronephrosis and acute respiratory distress syndrome (ARDS). During the current admission, she underwent ureteroscopy and replacement of DJ stent. Urine sample obtained via right-sided percutaneous nephrostomy (PCN) was sent to the microbiology laboratory

for culture and susceptibility testing. The urine culture yielded $>10^5$ CFU/ml of XDR *Klebsiella pneumoniae* (designated KP1a) susceptible to only colistin and tigecycline. In a repeat septic workup, the blood culture and PCN culture yielded PDR *K. pneumoniae* (KP1b) resistant to all antibiotics including colistin and tigecycline.

The patient was started on empirical intravenous (IV) meropenem 1g 8 hourly. Despite this therapy, she went into septic shock requiring inotropic support and necessity for intensive care unit (ICU) admission where IV amikacin 700 mg once daily was empirically added. Upon isolation of XDR *K. pneumoniae*, she was immediately transferred into an isolation room and strict contact precaution protocol was initiated. Intravenous colistin, 9 million IU loading dose, followed by 2 million IU 12 hourly for 21 days was added. The PCN was replaced with a new DJ stent. The patient remained in the ICU isolation room, responded very well to the therapy and was discharged home after 3 weeks. There was no recurrence of her infections at outpatient (OP) follow-up 4 weeks after discharge.

Case 2:

A 73-year-old diabetic man with considerable comorbidities; nephropathy, hypertension, dyslipidemia, osteoarthritis and immune thrombocytopenic purpura (on oral prednisolone), peripheral vascular disease, and bilateral chronic lower limb ischemia, underwent coronary artery bypass grafting for ischemic heart disease (multi-vessel disease) in February of 2017. He was on intermittent hemodialysis via right subclavian long-term hemodialysis catheter. At the admission on May 17, 2019, he presented with signs and symptoms of pulmonary edema and acute-on-chronic kidney disease. Shortly after admission, he developed severe shortness of breath requiring intubation with ventilator support and was shifted to the ICU. On the 3rd day post-intubation, he developed fever (Temp of 39ºC) and leukocytosis (WBC count of 15,000/µL). Samples of blood, urine and respiratory secretion were sent to the laboratory for investigations. Culture of endotracheal secretion yielded XDR *K. pneumoniae* (KP3) resistant to all antibiotics including colistin, aminoglycosides and the fluoroquinolones, except tigecycline (MIC=1.5 µg/ml). His rectal swabs were positive for XDR *K. pneumoniae* with the same susceptibility pattern as KP3.

He was put on contact isolation and empirically started on IV meropenem 1g 12-hourly. The antibiotic regimen was changed to IV colistin, 9 million IU loading dose, followed

by 2 million IU 12 hourly, nebulized colistin, 3 million IU daily, and IV tigecycline, 100 mg loading dose, followed by 50mg 12-hourly. There was remarkable improvement after 7 days on this therapy and was subsequently extubated and discharged to the ward on the same antibiotics which continued for a period of 14 days. He was discharged home hale and hearty. His out-patient follow-up was unremarkable.

Case 3:

This was an 85-year-old male patient, a known case of diabetes mellitus (DM), hypertension (HBP), old cerebrovascular accident (CVA) sustained in 2008, bedridden on naso-gastric tube feeding, urethral stricture on permanent Foley's catheter, benign prostatic hypertrophy (BPH) for which he underwent transurethral prostate resection in 2012, and normal pressure hydrocephalous. In addition, he also had a history of retinal detachment back in 2014. Six months before presentation, he was diagnosed as a case of myasthenia gravis in Jordan and was given IV immuno-globulin with slight improvement. He had recently developed bilateral pressure sores on the heels necessitating admission to our hospital on January 3, 2019.

The intervention given included debridement of his wound and was administered IV meropenem, 1g 8 hourly for 7 days. Subsequently, he was well enough to be discharged home. However, on February 10 2019, he was brought back to the emergency room (ER) de-saturated and semi-comatose. He was intubated and shifted directly to the ICU. IV norepinephrine bitartrate (levophed) 2 µg/kg/min and IV dopamine 10 µ/kg/min were started to maintain his blood pressure. After collecting appropriate specimens for sepsis work-up, he was started empirically on IV piperacillin-tazobactam, 4.5g 6 hourly and IV levofloxacin, 500 mg daily. On day 2 of admission to the ICU, the result of his blood culture revealed the growth of XDR *K. pneumoniae* (KP4) susceptible only to amikacin and tigecycline. The antibiotic regimen was changed to IV colistin 9 million, IU loading dose, followed by 4 million IU 12 hourly and IV tigecycline 100 mg loading dose followed by 50 mg 12 hourly.

He was isolated in a single room under contact precaution. On day 7 post-ICU admission, he showed some improvement and was transferred to the ward. However, while on the ward, his condition deteriorated, he developed disseminated intravascular coagulopathy (DIC), and went into cardiac arrest. All efforts to resuscitate him failed and he was pronounced dead a few hours after.

Case 4:

This was a 68-year-old patient, known hypertensive patient with uncontrolled DM. Past medical history revealed previous hemi-colectomy and ileostomy done in 2014 for colon carcinoma for which he was also treated with chemotherapy. He was admitted to our hospital on April 25, 2018 with complaints of swollen and tender right thigh accompanied with limitation of movement and fever (Temp: 39°C). Ultrasonography (US) of the affected limb showed ilio-psoas abscess extending to the right thigh.

The initial intervention was prompt incision and drainage of the abscess with the pus specimen sent to the microbiology laboratory for culture and susceptibility testing. He was started on empirical IV piperacillin-tazobactam, 4.5g 8 hourly. The pus yielded methicillin-sensitive *Staphylococcus aureus* (MSSA). As a result, IV clindamycin, 900 mg 8 hourly was added. There was an initial improvement followed by sudden drop in the blood pressure with accompanying abdominal pain but abdominal x-ray did not reveal any underlining cause. Repeat blood culture was negative. His blood pressure continued to drop requiring administration of vasopressors and transfer to the ICU. The next blood culture, taken on June 22 2018, yielded mixed infections with susceptible *K. aerogenes* and XDR *Escherichia coli* (EC1) susceptible only to amikacin and tigecycline. Strict contact precautions and isolation were promptly instituted. The treatment regimen was replaced by IV amikacin 15mg/kg 8 hourly plus IV tigecycline 100 mg loading dose, followed by 50mg 12 hourly. There was no clinical improvement and the patient passed away 48 hours after.

Case 5:

A 63-year-old diabetic patient with high BP, congestive cardiac failure (CHF), left ventricular hypertrophy and multiple myeloma was admitted to our hospital on January 12, 2018 with a 10-day history of shortness of breath that worsened on exertion, associated with orthopnea and productive cough. She had a past medical history of previous admission at an Indian hospital for chest infections caused by H1N1 Influenza A virus two months prior to presentation. On examination, she was afebrile with stable vital signs. Her chest examination revealed bilateral basal crepitations. Chest radiograph (CXR) ordered at this time showed bi-

lateral pleural effusion.

She was promptly started on diuretics and oral levofloxacin 750mg once daily. On day 2 of admission, she developed tachypnea and was put on bi-level positive airway pressure (BiPAP) therapy. Repeat CXR showed worsening of pleural effusion necessitating pleural tap. On day 3, the pleural fluid was reported as sterile by the microbiology laboratory. She was then seen by a consultant haematology/oncologist who prescribed interventional chemotherapy which was stoutly rejected by the patient family. On day 6, she developed high grade fever (Temp: 40°C). Hematological investigations revealed leukocytosis with a shift to the right (22,000 WBC/μL). IV meropenem, 1g 8 hourly was added to the levofloxacin. Her level of consciousness deteriorated but her family once again refused ICU admission. On day 7, she developed evidence of UTI and acute renal shut down requiring hemodialysis. Urine culture yielded significant growth of PDR *K. pneumoniae* (KP5) resistant to all antibiotics tested. She was isolated with strict contact precautions and put on inotropes and vasopressors for BP maintenance. Despite all the efforts, she succumbed to the ongoing sepsis due to PDR organism and passed away on day 8 of admission.

Case 6:

This 36-year-old patient was a known case of Down's syndrome, hiatus hernia, peptic ulcer disease, bronchial asthma (on nebulizer) and, in addition, had laparoscopic cholecystectomy done 3 years prior to presentation. There was history of multiple admissions to our hospital because of exacerbation of his asthma and community-acquired pneumonia (CAP) during the preceding 6 months. On this hospital visit on June 23 2019, he was admitted to the surgical ward for elective repair of hiatus hernia. Post-operatively, he was moved to the ICU for observation. Without appropriate microbiology/ infectious diseases consultation, he was started on empirical IV piperacillin-tazobactam 4.5g 8-hourly. On day 2 post-op, he vomited coffee-ground contents and a diagnosis of bleeding peptic ulcers was confirmed by upper GI endoscopy. A naso-jejunostomy tube was inserted.

On day 5 post-op, he developed septic shock requiring intubation and inotropic support via peripherally inserted central catheter (PICC) line. Full septic work-up was ordered and his antibiotic therapy changed to IV meropenem 1g 8 hourly. His blood culture taken from peripheral line yielded XDR *K. pneumoniae* (KP6) resistant to all antibiotics except tigecycline, trimethoprim-sulfamethoxazole and aminoglycosides. Meropenem was stopped and

IV trimethoprim-sulfamethoxazole 960 mg 12 hourly combined with IV gentamicin 5mg/kg/ day, in divided doses, 8-hourly, was commenced. He was isolated under strict contact precautions. There was remarkable improvement on this combination therapy and he was discharged to another hospital in USA for further non-infectious management.

Microbiology investigations:

Bacterial isolates:

All specimens were transported to the routine clinical diagnostic microbiology laboratory and processed according to the standard operational protocols (SOPs). Briefly, 10ml of blood was drawn from each patient as indicated and dispensed into 2 bottles of BD BACTEC 9240 (Microbiology System, Becton Dickinson, Massachusetts, USA) and incubated in BACTEC Blood Culture System (BD) for 24 h to 5 days at preset temperature of 37°C. Other specimens were inoculated onto appropriate selective and non-selective culture media according to instructions in the SOPs and incubated in air at 37°C for 24-48 hours or in the Anoxomat Anaerobic System (bioMérieux, Marcy, L'Étoile, France) that generated CO_2 (10%), H_2 (10%) and N_2 (80%), for 24 –hours to 7 days.

Representative isolates from these patients' specimens were stored at -80°C in Cryo-Bank beads (Mast Group Limited, Merseyside, UK) in the Anaerobe/Hospital Infection Reference Laboratory in the Department of Microbiology, Faculty of Medicine, Kuwait University. They were later retrieved, thawed and investigated for the genes mediating their resistance phenotypes by molecular characterization.

Re-isolation and identification of isolates:

The thawed bacterial suspensions were sub-cultured onto MacConkey agar (Oxoid, Basingstoke, UK) and blood agar (Oxoid). The inoculated plates were incubated in air at 37°C for 24 h. Representative colonies on the agar were then re-identified to species level by VITEK-2 ID System (bioMérieux, Marcy, L'Etoile, France).

Antibiotic susceptibility testing (AST):

AST was performed by determining the minimum inhibitory concentrations (MICs) of clinically relevant antibiotics using the E-test (bioMérieux) in accordance with the manufacturer's protocol, and by the agar dilution method where applicable (e. g. colistin susceptibility by CLSI) (18). In each test run, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *E. coli* ATCC 35218 were included as potency and media controls. Results were

interpreted according to the interpretative criteria of the Clinical and Laboratory Standards Institute (18), and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Guidelines for tigecycline and colistin (19).

Phenotypic and genotypic tests for detection of carbapenemase

The Modified-Hodge test and metallo-beta-lactamase (MBL) E-test were performed according to standard methods (20) to phenotypically detect metallo-beta-lactamases. Bacterial strains phenotypically MBL-positive were screened for the presence of genes mediating carbapenemase production, specifically bla_{VIM}, bla_{IMP}, bla_{KPC}, bla_{OXA-23}, bla_{OXA-48}, $bla_{OXA-181}$, bla_{NDM-1}, and bla_{NDM-5}, using multiplex PCR assay method with previously published primers (21-23), as well as by gene sequencing. Colistin resistance gene was investigated by multiplex PCR assay (24).

Definitions:

According to the European Centre of Disease Prevention and Control (ECDC) and Centre of Disease Control and Prevention (CDC), MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories; XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories while PDR is defined as non-susceptibility to all commercially available antimicrobial categories (25). Carbapenem-resistant Enterobacterales (CRE) was defined as isolate that showed decrease susceptibility to the carbapenems (ertapenem MIC > 0.5 µg/ml; imipenem, MIC > 1 µg/ml and/or meropenem, MIC > 1 µg/ml) regardless of carbapenem resistance (18).

Results:

The demographic profiles, underlying morbidities and empirical antibiotic therapy of the cases are summarized in Table 1. Three (50%) of the 6 patients had bloodstream infection (BSI) and 2 (33.3%) had UTI. There were 4 males and 2 females with male-to-female ratio of 2:1. Their ages ranged from 36 to 85 years (mean age of 60.7 years). Four (67%) of the 6 patients were diabetic. A total of 4 (67%) patients were treated with colistin, 3 (50%) with tigecycline, 2 (33.3%) with meropenem and 1 (16.7%) with trimethoprim-sulfamethoxazole.

As shown in Table 2, 3 (50%) of the 6 patients infected by XDR and PDR isolates died, and 3 (50%) survived and discharged home in good health. All but one of them were infected by *K. pneumoniae*. A total of 8 *K. pneumoniae* and 1 *E. coli* were isolated from the patients. The first patient was infected by the same phenotypically identical *K. pneumoniae* from urine, PCN and blood cultures.

Table 3 shows that all the clinical isolates were highly resistant to all the cephalosporins, fluoroquinolones, carbapenems, colistin and most of the aminoglycosides. The 3 blood isolates, KP4, KP6 and ECI, were susceptible to tigecycline with MICs of 2µg/ml, 2µg/ml and 0.38µg/ml, respectively. The results of multiplex PCR and sequencing showed that the isolates harbored multiple genes that mediated resistance, namely; bla_{NDM-1}, bla_{NDM-5}, bla_{OXA-48}, and $bla_{OXA-181}$ (Table 4), but bla_{VIM}, bla_{IMP}, and bla_{KPC} were not detected. All the colistin resistant isolates were negative for the presence of *mcr*-1 plasmid genes.

Discussion:

In this communication, we presented a series of six cases encountered in our hospital over one and half-year period with high mortality rate of 50%. Approximately, 83.3% of the patients were infected by MDR *K. pneumoniae* resistant to almost all the available antibiotics in our hospital formulary. These isolates were encountered in different clinical specimens including blood, urine and respiratory secretions. An interesting commonality was the history of previous multiple hospitalizations by all patients either in the same clinical center or different centers or even in different countries altogether.

Table 1: Characteristics of patients infected by multidrug-resistant microorganisms during hospital admission in Kuwait

S/N	Age (years)	Past medical history and previous hospital admission	Duration of current hospitalization before infection	Category of current admission	Current admitting diagnosis	Comorbidities	Empirical intervention
1	39	Insertion of Double-J stent; Kidney obstruction and hydronephrosis in the Surgical ward	3 days	ICU	Septic shock	Acute respiratory distress syndrome (ARDS)	Ureteroscopy, Inotropic support, IV meropenem 1g 12 hly and amikacin 500 mg 12 hly
2	73	Multiple hospital admissions, known case of DM, previous IHD with coronary bypass surgery. on hemodialysis	3 days	ICU	Pulmonary edema, acute-on-chronic renal failure, severe shortness of breath	Nephropathy, hypertension, dyslipidemia, osteoarthritis, immune thrombocytopenia purpura, bilateral chronic lower limb ischemia	Intubation with ventilator support, IV meropenem 1g 12 hly
3	85	Known case of DM, CVA, BPH, TUPR in 2012, normal pressure hydrocephalous, Myasthenia gravis. Previous hospital admission for bilateral pressure sores of the heels and debridement	2 days	ICU	Desaturation and loss of consciousness at home.	Low blood pressure, disorientation, urethral stricture, BPH	Intubation, IV levophed 2 mcg/kg/min, IV dopamine 10 mcg/kg/min, IV piperacillin-tazobactam 4.5 mg 6 hly, IV levofloxacin 500 mg daily
4	68	Known HBP, DM, previous hemicolectomy and ileostomy for colon cancer, chemotherapy.	53 days	Surgical ward → ICU	Ilieo-psoas abscess, fever, intra-abdominal pain.	Uncontrolled DM, HBP	Ultrasonography, incision and drainage, IV piperacillin-tazobactam 4.5g 8 hly, IV clindamycin 900mg 8 hly. Later, IV amikacin 15mg/kg 8 hly, IV tigecycline 100 mg start and 50 mg x 12 hly.
5	63	DM, HBP, CCF, multiple myeloma, previous hospital admission in India, H1N1 flu chest infection	6 days	ICU	Bilateral pleural effusion, UTI, acute renal failure	DM, HBP, high fever (T=40ºC), leukocytosis	Per oral levofloxacin 750 mg od, bi-level positive airway pressure, IV meropenem 1g 8 hly
6	36	Known case of Down's syndrome, hiatus hernia, PU, asthma, CAP, cholecystectomy at previous hospital admission 3 years back, history of multiple hospital admissions	6 days	Surgical ward → ICU	Hiatus hernia	PU, bronchial asthma	Naso-jejunostomy tube, IV piperacillin-tazobactam 4.5 g 8 hly, later IV meropenem 1g 8 hly

DM=diabetes mellitus; CCF=congestive cardiac failure; HBP=high blood pressure; IHD=ischemic heart disease; CVA=cerebrovascular accident; TUPR=transurethral prostatic resection; BPH=benign prostatic hypertrophy; PU=peptic ulcer; CAP=community-acquired pneumonia; IV=intravenous; ICU=intensive care unit; od=once daily; S/N=serial number

Table 2: Characteristics of patients' pan-resistant and extensively-resistant isolates, site of infection, antimicrobial therapy and outcome of infections during hospital admission

S/N of patient (isolate designation)	Isolate	Site of infection	Total duration of hospital admission	Prior antibiotic therapy	Antimicrobial treatment/duration	Outcome
1 (KP1a)	*Klebsiella pneumoniae*	Urine culture	30 days	IV meropenem 1g 8 hly	IV meropenem 1g 8hly + IV amikacin 700 mg OD. Later, IV colistin 9 million IU loading dose followed by 2 million IU 12 hly + IV meropenem 1g 8 hly x 21 days.	Survived
(KP1b)	*Klebsiella pneumoniae*	PCN culture				
(KP1c)	*Klebsiella pneumoniae*	Blood culture				
2 (KP2a)	*Klebsiella pneumoniae*	Respiratory secretion	21 days	IV meropenem 1g 8 hly	IV colistin 9 million IU loading dose, followed by 2 million IU 12 hly + nebulized colistin 2 million IU od + IV tigecycline 100 mg start followed by 50 mg 12 hly x 21 days	Survived
(KP2b)	*Klebsiella pneumoniae*	Rectal swab				
3 (KP3)	*Klebsiella pneumoniae*	Blood culture	14 days	IV piperacillin-tazobactam 4.5 g 6 hly	IV colistin 9 million IU start, followed by 2 million IU 12 hly + IV tigecycline 100 mg loading dose followed by 50 mg 12 hly	Expired
4 (EC1)	*Escherichia coli*	Blood culture	58 days	IV piperacillin-tazobactam 4.5g 8 hly, IV clindamycin 900 mg tid	IV amikacin 15/kg 8 hly + IV tigecycline 100 mg loading dose, followed by 50 mg 12 hly	Expired
5 (KP4)	*Klebsiella pneumoniae*	Urine culture	10 days	Oral levofloxacin 750 mg OD	IV meropenem 1g 8 hly + IV levofloxacin 750 mg/150 ml	Expired
6 (KP5)	*Klebsiella pneumoniae*	Blood culture	14 days	IV piperacillin-tazobactam 4.5 g 8 hly, IV meropenem 1g 8 hly	IV trimethoprim-sulfamethoxazole 960 mg 12 hly + IV gentamicin 5 mg/kg/day in divided dose 8 hly	Survived

Kp1a=*Klebsiella pneumoniae* isolated from urine culture of case no 1; Kp1b=*K. pneumoniae* isolated from percutaneous nephrostomy tube culture of case no 1; Kp1c=*K. pneumoniae* isolated from blood culture of case no1; Kp2a=*K. pneumoniae* isolated from respiratory secretion of case no 2; Kp2b=*K. pneumoniae* isolated from rectal swab of case no 2; Kp3=*K. pneumoniae* isolated from blood culture of case no 3; EC1=*E. coli* isolated from blood culture of case no 4; Kp4=*K. pneumoniae* isolated from blood culture of case no 5; Kp5=*K. pneumoniae* isolated from blood culture of patient no 6; S/N=serial number; tid=three times a day

The *K. pneumoniae* isolates harbored multiple genes that encoded carbapenemases production such as NDM and OXA classes which sequence analysis revealed to be NDM-1, NDM-5, OXA-48 and OXA-181. Selection of appropriate antimicrobial agents for the treatment of infections caused by these CRE strains was a clinical challenge. Despite concerns about being nephrotoxic and neurotoxic, the use of old antibiotics, such as colistin, as a 'last resort' treatment have come to the forefront (26). Unfortunately, colistin is being overused, or used with suboptimal dosing regimen in some settings, including our hospital, and this has inadvertently led to growing resistance of Gram-negative bacteria to this life-saving agent (26,27).

All but one of the clinical isolates in our study were phenotypically resistant to colistin. It is conceivable that this was, in part, due to the overuse of this agent in our hospital. PCR for *mcr* gene mediating resistance to colistin was negative in all our isolates, a finding that is not surprising as most cases of plasmid-mediated *mcr*-1 genes have been found mainly in *E. coli* isolates.

Table 3: Minimum inhibitory concentrations of the antimicrobial agents tested against the clinical isolates

Antibiotic (breakpoint in µg/ml)	KP1a/KP1b/KP1c (MIC µg/ml)	KP2a/KP2b (MIC µg/ml)	KP3 (MIC µg/ml)	EC1 (MIC µg/ml)	KP4 (MIC µg/ml)	KP5 (MIC µg/ml)
Cefotaxime (4)	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)
Ceftazidime (4)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)
Cefepime (16)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)
Piperacillin/tazobactam (128/4)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)
Ertapenem (2)	R (>32)	R (>32)	R (>32)	R (>256)	R (>32)	R (>256)
Imipenem (4)	R (>32)	R (>32)	R (8)	R (>256)	R (>32)	R (>32)
Meropenem (4)	R (>32)	R (>32)	R (>32)	R (>256)	R (>32)	R (32)
Amikacin (64)	R (>256)	R (>256)	S (4)	R (>256)	S (6)	S (16)
Gentamicin (16)	R (768)	R (>1024)	R (768)	R (512)	R (512)	S (1)
Ciprofloxacin (1)	R (>32)	R (24)	R (>32)	R (>32)	R (>32)	R (32)
Levofloxacin (2)	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)	R (32)
Tigecycline	R (6)	S (1.5)	S (2)	R (3)	S (0.38)	S (2)
Colistin	R (24)	R (12)	R (12)	R (24)	R (4)	R (12)
Trimethoprim/sulfamethoxazole (4)	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)	S (<20)
Rifampicin	R	R	R	R	-	-
Chloramphenicol (32)	R (32)	R (32)	R (>256)	R (48)	R (32)	-

Kp1a = *Klebsiella pneumoniae* isolated from urine culture of case no 1; Kp1b = *K. pneumoniae* isolated from percutaneous nephrostomy tube culture of case no 1; Kp1c = *K. pneumoniae* isolated from blood culture of case no1; Kp2a = *K. pneumoniae* isolated from respiratory secretion of case no 2; Kp2b= *K. pneumoniae* isolated from rectal swab of case no 2; Kp3 = *K. pneumoniae* isolated from blood culture of case no 3; EC1 = *E. coli* isolated from blood culture of case no 4; Kp4 = *K. pneumoniae* isolated from blood culture of case no 5; Kp5 = *K. pneumoniae* isolated from blood culture of patient no 6; MIC = Minimum inhibitory concentration µg/ml*;* S = sensitive; R = resistant. Rifampicin disc diffusion method was used as there was no E-test available.

Table 4: Molecular characterization of the genes mediating carbapenemase production in the XDR/PDR isolates.

Serial number	Organism	*bla*NDM	*bla*KPC	*bla*OXA	*bla*VIM	*bla*IMP	*bla*mcr-1
1	KP1a/KP1b/KP1c	*bla*NDM-5	-	-	-	-	-
2	KP2a/KP2b	*bla*NDM-1	-	*bla*OXA-48	-	-	-
3	KP3	-	-	*bla*OXA-48	-	-	-
4	EC1	-	-	*bla*OXA-181	-	-	-
5	KP4	*bla*NDM-1	-	-	-	-	-
6	KP5	*bla*NDM-5	-	*bla*OXA-181	-	-	-

Kp1a = *Klebsiella pneumoniae* isolated from urine culture of case no 1; Kp1b = *K. pneumoniae* isolated from percutaneous nephrostomy tube culture of case no 1; Kp1c = *K. pneumoniae* isolated from blood culture of case no1; Kp2a = *K. pneumoniae* isolated from respiratory secretion of case no 2; Kp2b= *K. pneumoniae* isolated from rectal swab of case no 2; Kp3 = *K. pneumoniae* isolated from blood culture of case no 3; EC1 = *E. coli* isolated from blood culture of case no 4; Kp4 = *K. pneumoniae* isolated from blood culture of case no 5; Kp5 = *K. pneumoniae* isolated from blood culture of patient no 6.

Another class of new antibiotic is tige-cycline, a minocycline derivative known as gly-cylcycline, which is being exploited and over-used in many hospitals globally. It has a broad-spectrum activity against many Gram-positive, Gram-negative as well as anaerobic pathogens (28,29). It is usually prescribed as part of a combination therapy against CRE because of its good *in vitro* activity against many MDR and XDR Enterobacterales (30,31). In our series, two isolates were resistant to tigecycline thus reducing the treatment option. Even in isolates that were susceptible to tigecycline, the thera-peutic use of this drug has a serious limitation because of its poor achievable serum and urine concentrations according to many studies (28, 32).

The first 3 patients in our series were

treated with combination therapies involving colistin and tigecycline (2 cases) or colistin and meropenem (1 case). Two of these patients survived. Colistin and tigecycline have been used as first line agents for the treatment of infections caused by MDR Enterobacterales. However, there are uncertainties with respect to their efficacy as the mortality rates from these septic episodes still remain high. On the other hand, 2 patients (case nos. 4 and 5) not treated with combinations involving colistin died. The third case (case no. 6) was on tri-methoprim-sulfamethoxazole and his condition improved remarkably by the time his family requested for his discharge to seek treatment abroad. Although recently, some new and combination agents with activity against MDR organisms and CRE, such as ceftazidime-avibactam, ceftolozane-tazobactam, meropenem-vaborbactam, imipenem-cilastin-relebactam, plazomicin, eravacycline and cefiderocol, have been approved for clinical use or are at the final stages of development, none of these agents is available in our country at this time.

It is of interest to note that the first case who had UTI and bloodstream infections with different XDR and PDR *K. pneumoniae* strains survived on prolonged colistin plus meropenem combination therapy. For all the cases, bundles interventions including enhanced environmental cleaning, contact precautions as well as antimicrobial stewardship were instituted promptly. As Enterobacterales are among the leading causes of healthcare associated infections, early identification of resistant bacteria is of paramount importance to the success of infection control efforts. In our hospital, active surveillance of patients helped to improve infection control by detecting colonization and preventing horizontal spread by these dangerous MDR pathogens. The CDC guidelines for surveillance, recommends the use of active surveillance in outbreaks, and that even non-endemic acute care facilities should review all clinical cultures within the last 6-12 months for previously unrecognized carbapenemase-producing Enterobacterales (CPE) (33). We believe that the aggressive infection control efforts in our hospital, including the bundle interventional guidelines introduced at the appropriate time, have been effective in decreasing rates of infections with CPE.

Currently, the therapeutic options for highly resistant carbapenemase-producing organisms in our hospital are limited. Prudent antimicrobials use becomes increasingly important as we are faced with little or no bright future for procurement of effective alternate drugs. Information on how to treat infections with CPE is still surprisingly limited, in spite of rapidly increasing prevalence of these organisms. Comprehensive clinical studies of the main therapeutic options, broken down by pathogens, enzymes and clinical syndrome, are definitely lacking. Clinicians need accurate susceptibility data to provide effective therapy.

Conclusion:

Rapid routine molecular detection is essential to optimize therapy, improve outcomes and limit the spread of CRE through aggressive infection control measures, including screening of potentially colonized high-risk patients (31). Even when we eventually introduce new antimicrobial agents into the hospital armamentarium, antimicrobial stewardship will play crucial important role, as these drugs will come with their own strengths and caveats.

Contributions of authors:

All authors made substantial contributions to all the following; (i) the conception and design, or acquisition of data, or analysis and interpretation of data; (ii) drafting the article, or revising it critically for important intellectual content; and final approval of the revision to be submitted. All authors have approved the final manuscript.

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