Polish Annals of Medicine

Journal homepage: https://www.paom.pl

Septic shock caused by community-acquired urinary tract infection caused by *Klebsiella pneumoniae* ESBL+: A case report

Małgorzata Braczkowska[1], Lidia Glinka[1] ⓘD, *Marcin Mieszkowski[1],*
Bułat Tuyakov[1], Aleksandra Gutysz-Wojnicka[2]

[1] *Department of Anaesthesiology and Intensive Care, School of Medicine, Collegium Medicum,*
University of Warmia and Mazury in Olsztyn, Poland
[2] *Department of Nursing, School of Public Health, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Poland*

ARTICLE INFO

ABSTRACT

Introduction: Septic shock is defined as a life-threatening organ failure caused by an abnormal response of the body to infection. Urinary tract infections (UTIs) constitute about 10%–20% of all community-acquired infections and about 40%–50% of hospital-acquired infections. In patients with impaired immunity they may lead to sepsis. Strains of *Klebsiella pneumoniae* are often multidrug resistant, and therapeutic chances are limited where they occur.

Aim: The aim of this paper is to discuss the most recent guidelines in diagnosing and treating sepsis, referring to a clinical case report.

Case study: The study presents a case of septic shock in a 44-year-old female patient in a community-acquired UTI caused by *K. pneumoniae* extended-spectrum β-lactamases (ESBL+).

Results and discussion: The course of septic shock proved fatal. As the stay in the intensive care unit (ICU) was short, this precluded implementing full diagnostic procedures and identifying the source of infection. A post mortem examination was performed to establish the cause of death and aetiology of the infection.

Conclusions: *K. pneumoniae* ESBL+ has become a growing epidemiological problem in Poland and all over the world. This pathogen increasingly often leads to community-acquired infections and its multidrug resistance makes the applied therapies ineffective. Diabetes, one of the modern lifestyle diseases, impairs resistance and accelerates rapidly progressing septic shock with multiple organ failure. Late diagnosis of sepsis, because of considerable metabolic and cellular changes, brings about tragic results. Despite implementing new diagnostic methods and therapies, the mortality rate in sepsis still remains very high.

Corresponding author: Lidia Glinka, Anaesthesiology and Intensive Care Clinical Ward, Clinical University Hospital in Olsztyn, Department of Anaesthesiology and Intensive Care, University of Warmia and Mazury in Olsztyn, Warszawska 30, 10-082 Olsztyn Poland. Tel. +4889 534 54 08.
E-mail address: lidka.glinka@gmail.com.

1. INTRODUCTION

The term 'sepsis' had already known in antiquity and then meant 'decaying organic matter' or a condition in whose course tissues are affected by the purulent process and which leads to an inauspicious end. In 1991, at a Consensus Conference in the USA a number of definitions and criteria referring to sepsis were agreed upon (SEPSIS-1). Since then, the **definition and guidelines for the management of sepsis** have been modified on numerous occasions in line with medical data based on scientific research (evidence based medicine – EBM).[1]

Pneumonia is the most common cause of sepsis, followed by peritonitis and urinary tract infections (UTIs).

UTIs constitute about 10%–20% of all community-acquired infections and 40%–50% of hospital-acquired infections. Considering their severe consequences, such as renal failure, hypertension and a generalized infection in patients with impaired resistance, they pose a **serious clinical problem.**[2]

Klebsiella pneumoniae is a highly destructive Gram-negative bacterium of *Enterobacteriaceae* family. It was first described by Friedländer in 1883, and it constitutes one of the most common causes of hospital-acquired infections, and recently also community-acquired infections as well. In natural conditions, it occurs in the digestive tract, in the nasal-pharyngeal cavity and on the skin.[3,4] In the treatment of infections caused by extended-spectrum β-lactamases (ESBL⁺) bacteria, the antibiotics of choice are carbapenems. Abuse of this group of drugs led to the creation in *Enterobacteriaceae*, new β-lactamases that hydrolyse carbapenems, such as metal-β-lactamase (MBL) and *K. pneumoniae* carbapenemase. Emergence is the biggest problem strains in which the coexistence of β-lactam ESBL, carbapenemase is observed and other resistance mechanisms that cause lack of sensitivity to fluoroquinolones or aminoglycosides. *Enterobacteriaceae* producing carbapenemase have been reported in Poland since 2012, but **in 2016 a sharp increase in the number** of colonized and infected patients was noticed. In total, in the first three quarters of 2017, a record number of New Delhi *K. pneumoniae* (NDM) isolates (+) was confirmed (*n* = 2512), despite the fact that from July 1, 2017 restrictions on the acceptance of strains from the area of Warsaw and Mazowieckie voivodeship were introduced. Currently, a national epidemic is observed, molecular studies performed for a large group of strains have shown the spread of *K. pneumoniae* NDM in Poland belonging to the pandemic ST11 clone.[5] Non-hospital-acquired infections most often affect people with an impaired immune system, burdened with chronic diseases, such as diabetes.[6–10]

The collected data suggest that about 171 million people in the world suffer from diabetes, out of which 50% remain undiagnosed due to lack of symptoms.[11] Despite the fact that diabetes is the most common concomitant disease, the number of clinical and experimental studies on its effect on the course of sepsis is still scanty.[12–16]

2. AIM

The aim of this paper is to discuss the most recent guidelines in diagnosing and treating sepsis, referring to a clinical case report.

3. CASE REPORT

A 44-year-old female patient was admitted to the ICU due to disorders of consciousness and suspected septic shock. On the day prior to the admission, she stayed in the internal medicine department, to which she had been admitted because of nausea, vomiting, stomach ache, high **temperature** and cough. These symptoms had lasted for a few days. Her medical history included: badly controlled diabetes (the patient had not taken insulin for 2 weeks before **hospitalization**, glycaemia on admission 578 mg/dL, glycated haemoglobin 16.52%), history of acute pancreatitis, allergy to ciprofloxacin. For a few days before admission, the **patient** had taken amoxicillin with clavulanic acid prescribed by her general practitioner because of fever and cough. At hospital, empiric therapy was complemented with ceftriaxone, amikacin and clarithromycin. The laboratory tests showed high inflammatory parameters: CRP > 477 mg/L, PCT 113 ng/mL. Urinalysis detected glucose, protein, ketones, nitrites; numerous leukocytes and bacteria in urine sediment. In the chest X-ray no inflammatory lesions were found. In the computed tomography scan of the head no evident foci in the central nervous system were found, despite the occurrence of positive meningism in the clinical tests. The ultrasound examination of the abdominal cavity revealed diminished corticomedullary differentiation in the left kidney, a cyst in the head of the pancreas, and cholelithiasis, with suspected inflammation of the gall bladder. The patient was consulted by a neurologist and a surgeon, who recommended further observation.

After less than 20 hours of her hospital stay in the internal medicine department, due to deteriorating condition, the patient was transferred to the ICU.

On admission to the ICU, in the physical examination it was found that the patient had disorders of consciousness, verbal contact was limited, pupils mid-dilated with sluggish pupillary response, nuchal rigidity was found in the neurological examination. The patient was not intubated, oxygen was delivered via a face mask, with tachypnea about 30 breaths/min, SpO₂ = 98%. During auscultation of the chest, vesicular sound was symmetrical, with discrete crackling in the field of auscultation. The circulatory system was supported with an infusion of noradrenaline, the blood pressure was 80/40 mmHg, heart rate 120 bpm with frequent ventricular extra systoles. The skin was pale, extremities cold, capillary refill time over 5 s. Anuria was found. The abdomen was arched below the level of the chest, on palpation non-painful, with peristalsis, detectable resistance under the left rib arch. The temperature on admission to the ICU was 36.8°C.

The patient was immediately intubated and mechanical ventilation in line with the strategy of lung protective ventilation was introduced. The administered therapies included: analgosedation, empiric broad-spectrum antibiotic therapy (meropen, vancomycin), prophylaxis of venous thromboembolic disease, prophylaxis of stress ulcers, restoring the fluid and electrolyte balance and acid/alkaline balance; specimens for culture were obtained. The patient was monitored hemodynamically in an extended manner by means of **constantly measuring the cardiac output with thermodilution**. The thermodynamic profile showed intravascular volume depletion, low cardiac output and low systemic peripheral resistance. The laboratory tests determined indicators of severe septic shock with multiple organ failure: high inflammatory parameters (CRP 477 mg/L, PCT 114 ng/mL, leukopenia $3.7 \times 10^3/\mu L$), blood clotting disorders with thrombocytopenia (APTT 44.9, INR 1.6, PLT $75 \times 10^3/\mu L$, fibrinogen > 900 mg/dL), increased parameters of renal failure (urea 93 mg/dL, creatinine 2.8 mg/dL, eGFR 19.9), severe metabolic acidosis (pH = 7.21, HCO_3^- 10.3, pCO_2 27.3, BE −15.4) with lactate level over 7, hyperglycaemia (311 mg/dL), hypertriglyceridemia, hypoalbuminemia (4.6 g/dL), increased level of bilirubin (1.7 mg/dL), heart failure (NT pro-BNP 40737 pg/mL,) rapidly increasing level of troponin T (from 0.677 to 3.2 in 2 h), features of anterior myocardial infarction in the electrocardiogram.

Septic and cardiogenic shock was diagnosed and steps were immediately taken in order to identify the source of infection. Because of the progressing hypotension, in line with introducing fluid therapy, the dose of noradrenaline was increased, accompanied by a continuous intravenous infusion of adrenaline and steroids. The patient was consulted by a surgeon – on the basis of the ultrasound imaging and physical examination (pain in the right hypochondrium, suspected acute abdomen), she was qualified to diagnostic laparotomy subsequent to stabilising the general condition. After gynaecological consultation, lesions in the reproductive system were excluded.

In the echocardiogram, the following findings were made: akinetic aneurism of the apex of the heart, akinesis of middle segments of the lower anterior wall and septum, hyperkinetic lateral wall, lower latter and base segments of the anterior and lower wall, EF 31%–43%, the inferior vena cava dilated 24 mm with reduced respiratory variation, suspected takotsubo stress cardiomyopathy. A computed tomography scan of the chest and abdominal cavity was planned, yet it could not be performed due to lack of hemodynamic stability of the patient. In the 4th h after admission to the ICU, the patient died after ineffective resuscitation (cardiac arrest in asystolia).

Since the cause of cardiogenic and septic shock was not determined and completing the diagnostic examination was not possible (no chance to perform lumbar puncture because of thrombocytopenia and disorders of blood clotting, no results of cultures), it was decided to subject the patient's body to post mortem examination.

The cultures obtained in the ICU whose results were available after the patient's death did not reveal an increase in blood bacteria, while in urine and bronchial secretion *K. pneumoniae* producing enzymes of extended spectrum of activity was found (*K. pneumoniae* ESBL$^+$). The antibiogram revealed resistance of the pathogens to amoxicillin with clavulanic acid, which the patient had taken before admission to hospital.

The post mortem determined that the direct cause of death consisted in multiple organ failure in the course of septic shock caused by acute pyelonephritis. Microscopic findings included: oedema of the brain, passive hyperaemia and oedema of the lungs, myocarditis, acute pyelonephritis with abscesses in the renal parenchyma and renal infarctions, inflammatory infiltration in the liver with intrahepatic bile duct obstruction, chronic pancreatitis. Cholecystitis was not confirmed.

4. DISCUSSION

In line with the 2017 guidelines of the international team of experts for management of sepsis (Surviving Sepsis Campaign – SSC), it can be said that the patient was in septic shock, defined as 'a form of sepsis with circulatory, metabolic and cellular abnormalities which are associated with a greater death risk.'

Septic shock is an emergency condition and requires undertaking instantaneous anti-shock treatment with fluid resuscitation and implementing vasoactive medication, with both invasive and non-invasive haemodynamic parameters monitoring. It is recommended to use dynamic indicators (e.g. echocardiogram, cardiac output measurements) in order to predict response to the administered fluids.[17]

The diagnostic procedure includes obtaining proper material for microbiological tests before implementing anti-microorganism treatment. Antibiotic therapy should be implemented up to 1 h after diagnosing sepsis. Empiric introduction of at least 1 broad-spectrum antibiotic should take into consideration its activity against all most likely aetiological factors. In the initial stage of the septic shock treatment it is recommended to apply empiric combination therapy with at least 2 antibiotics from different groups. The next diagnostic step is to control the focus of the infection and to undertake the necessary intervention as soon as possible.[17]

In the presented case, after admission to the ICU, all the criteria were met, apart from the one concerning diagnosing sepsis, as the patient had been admitted to the unit with an already developed septic shock and multiple organ failure. The starting point of the infection remained unclear due to numerous non-specific clinical symptoms and overlapping secondary dysfunctions of multiple organs.

The patient received fluid therapy, catechol amines, steroids, albumins and empiric antibiotic therapy. The glycaemia control protocol was introduced. The diagnostic process aimed at identifying the source of sepsis was commenced (laboratory tests, cultures, imaging, specialist consultations); however, it was not completed because of extremely

rapid progression of the disease. The patient was monitored in an extended manner, including the recommended invasive methods of cardiac output measurements. Central venous, arterial and dialysis cannulation procedures were performed. However, the course of treatment presented here was implemented too late. It seems that septic shock was then already in its irreversible stage. The sequential organ failure assessment (SOFA) score appears to be a useful tool in diagnosing septic shock. Yet it is concerned mainly with patients of ICUs, since ventilation parameters are among its criteria. The criterion of diagnosing sepsis consists in a rapid change in the score by 2 or more points with a concomitant suspicion of infection.

In community-acquired cases and outside the ICU, it is easier to apply the modified scale, the so called quick SOFA (qSOFA), according to which sepsis is suspected on the basis of at least 2 points.[17]

Experts' recommendation to use SOFA scores concerns sepsis, while when it comes to predicting the death risk – simplified acute physiology score 3 (SAPS3), seems to be more applicable. It takes into consideration numerous other factors which have effect on predicting mortality, among others: history of a surgical procedure, number of days outside the ICU and the place from which the patient was admitted, their age, mode of admission (planned, emergency), and others.

The patient received 15 points in the SOFA score and 3 points in the qSOFA score, which means that the risk of septic shock was high, with the predicted mortality over 80%. In the SAPS3 the patient received 83 points, with the predicted mortality of 91%. The level of lactates on admission to the ICU exceeded 7 mmol/L, which is known to constitute an independent prognostic factor in sepsis.

Empiric therapy did not improve the patient's condition: *K. pneumoniae* proved to be resistant to amoxicillin with clavulanic acid (taken by the patient at home) and ceftriaxone (administered on the first day of hospital stay). In UTIs, the first line treatment is fluoroquinolones, among others ciprofloxacin, to which the patient was allergic; the next step is II/III generation cephalosporin, e.g. Ceftriaxone, which was administered, yet the detected pathogen proved resistant to this medicine.

The strains of *K. pneumoniae* are often multidrug resistant (MDR), and in treating infections their therapeutic effectiveness is limited. One of the most common mechanisms of resistance in *K. pneumoniae* consists in producing β-lactamases of the ESBL type. The first strain of this phenotype was described in 1983, and since then it has been the most significant diagnostic, clinical and epidemiological problem in the ICU.[18–21] This is caused because new variants of ESBL originate; their range of activity is often wider, and they are resistant to inhibitors of β-lactamases. Initially, the strains of this phenotype had been resistant to penicillins and cephalosporins of earlier generations. In the course of evolution of β-lactamases, the strains which were capable of producing them have proved to be increasingly resistant to all β-lactam antibiotics apart from carbapenems. This is

also influenced by the fact that **different resistance mechanisms overlap**. Treating infections caused by *K. pneumoniae* ESBL[+] is becoming increasingly costly. Targeted antibiotic therapy does not guarantee curing the disease, and hospital-acquired infections with ESBL[+] strains are often burdened with high mortality.[5]

An additional problem is caused by the aforementioned diversity of these enzymes and easy transfer not only within strains of one species but also within one family or different groups of bacteria. Nowadays, 300 variants of β-lactamases of ESBL are known. Strains of species of *Enterobacteriaceae* family producing β-lactamases of the ESBL type are **isolated most often**.[22]

Although this mechanism conditions resistance to β-lactamases, one needs to remember that the ESBL-encoding plasmid contains also genes conditioning resistance to antibiotics from other groups, that is aminoglycosides, quinolones, tetracyclines. In such a situation, strains determined as MDR are detected.[22]

In the described case, diabetes constituted a significant risk of infection.

Irrespective of age, hypertension, dyslipidaemia and coronary thrombosis, diabetes increases the risk of heart failure. Irrespective of the traditional division of diabetes into two types, both **forms may lead to serious cardiovascular complications**, the so called diabetic cardiomyopathy with diastolic dysfunction and, in most cases, with preserved systolic function in the course of lipotoxicity.[23] In physiological conditions, continuous systolic function of the heart depends on appropriate supplies of energy which comes from carbohydrates (30% of ATP production) and fatty acids oxidation (70% of ATP production), and to a lesser extent from ketones and amino acids. In diabetes type 1 and 2, glucose uptake, glycolysis and pyruvic acid oxidation are impaired. Additionally, a lack of insulin predisposes the body to undergoing lipolysis and releasing free fatty acids from the adipose tissue; in such conditions the heart very often adapts to using fatty acid as the main source of energy (ATP production), and if this is a chronic process, it facilitates development of cardiomyopathy. Accumulation of fatty acids has effect on using glucose on many stages, leading to faulty uptake and oxidation of glucose stimulated by insulin.[24–25]

Diabetic patients are more susceptible to infections, for instance of lower respiratory tract and **urinary tract; irrespective** of the type (1 or 2) of diabetes the risk of blood infection is higher. Insulin has a strong anti-inflammatory effect, while hyperglycaemia has a strong inflammatory influence, both in vitro as well as in vivo. Concentrations of circulating inflammatory cytokines, such as CRP and IL-6, are higher in type 1 and 2 diabetic patients, especially in middle-aged women with type 2 diabetes. It has been proved that there is a higher risk of organ dysfunction in the course of sepsis if diabetes has occurred prior to sepsis. Comparing septic patients with and without diabetes, it can be concluded that diabetic patients less often developed pneumonia, while UTIs occurred significantly more frequently.[24-25]

The patient did not receive immunoglobulins since their effectiveness in treating sepsis has not been confirmed as of yet.

5. CONCLUSIONS

Klebsiella pneumoniae ESBL+ constitutes an increasingly serious epidemiological problem in Poland and in the whole world. This pathogen is more and more frequently a cause of community-acquired infections and its MDR often leads to a therapeutic failure. Diabetes, which is a lifestyle disease, undoubtedly leads to impaired immunity and rapid development of septic shock with multiple organ failure. Diagnosing sepsis too late brings tragic effects which are due to advanced metabolic and cellular abnormalities.

Despite implementing new diagnostic methods and therapies, mortality in sepsis still remains very high.

Conflict of interest
None declared.

Funding
None declared.

Ethics
None declared.

References
1. Kübler A, Nestorowicz A, Gaszyński W. Defeat sepsis. *Anestezjol Intens Ter*. 2004;4:304–309 [in Polish].
2. Chmielewska SJ, Fiedoruk K, Daniluk T, Ściepuk M, Kaczmarzyk D, Leszczyńska K. Significance of uropathogenic strains of *Escherichia coli* (UPEC) in the pathogenesis of urinary tract infections. *Post Mikrobiol*. 2016;55(1):45–56 [in Polish].
3. Ravichitra KN, Hema Prakash P, Subbarayudu S, Sreenivasa Rao U. Isolation and antibiotic sensitivity of *Klebsiella pneumoniae* from pus, sputum and urine samples. *Int J Curr Microbiol App Sci*. 2014;3(3):115–119.
4. Sękowska A, Gospodarek E, Kusza K. The prevalence of infections and colonisation with *Klebsiella pneumoniae* strains isolated in ICU patients. *Anaesthesiol Intensive Ther*. 2014;46(4):280–283. https://doi.org/10.5603/AIT.2014.0045.
5. National Reference Center for Drug Susceptibility Systemic. Report of the National Reference Center for Antimicrobial susceptibility Occurrence of *Enterobacteriaceae* (*Klebsiella pneumoniae*) producing carbapenem New Delhi type in Poland in the first quarter of 2017. Warszawa; 2017. http://korld.edu.pl/pdf/NDM_Raport_I_kwartal_2017-05-07-1.pdf. Accessed: May 23, 2018 [in Polish].
6. Koh GC, Peacock SJ, van der Poll T, Wiersinga WJ. The impact of diabetes on the pathogenesis of sepsis. *Eur J Clin Microbiol Infect Dis*. 2012;31(4):379–388. https://doi.org/10.1007/s10096-011-1337-4.
7. Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. *Lancet Infect Dis*. 2009;9(12):737–746. https://doi.org/10.1016/S1473-3099(09)70282-8.
8. Fine MJ, Smith MA, Carson CA, et al. Prognosis and outcomes of patients with community aquired pneumonia. A meta-analysis. *JAMA*. 1996;275(2):134–141.
9. Thomsen RW, Hundborg HH, Lervang HH, Johnsen SP, Schønheyder HC, Sørensen HT. Diabetes mellitus as a risk and prognostic factor for community-acquired bacteremia due to enterobacteria: a 10-year, population-based study among adults. *Clin Infect Dis*. 2005;40(4):628–631. https://doi.org/10.1086/427699.
10. Falangas ME, Alexiou VG, Giannopoulou KP, Siempos II. Risk factors for mortality in patients with emphysematous pyelonephritis: a meta-analysis. *J Urol*. 2007;178(3 Pt 1):880–885. https://doi.org/10.1016/j.juro.2007.05.017.
11. Osuchowski MF, Craciun FL, Schuller E, Sima C, Gyurko R, Remick DG. Untreated type 1 diabetes increases sepsis-induced mortality without inducing a prelethal cytokine response. *Shock*. 2010;34(4):369–376. https://doi.org/10.1097/SHK.0b013e3181dc40a8.
12. Esper AM, Moss M, Lewis CA, Nisbet R, Mannino DM, Martin GS. The role of infection and comorbidity: Factors that influence disparities in sepsis. *Crit Care Med*. 2006;34(10):2576–2582. https://doi.org/10.1097/01.CCM.0000239114.50519.0E.
13. Brun-Buisson C, Doyon F, Carlet J, et al. Incidence, risk factors, and outcome of severe sepsis and septic shock in adults. A multicenter prospective study in intensive care units. *JAMA*. 1995;274:968–974.
14. Vincent JL, Preiser JC, Sprung CL, Moreno R, Sakr Y. Insulin-treated diabetes is not associated with increased mortality in critically ill patients. *Crit Care*. 2010;14(1):R12. https://doi.org/10.1186/cc8866.
15. Carton JA, Maradona JA, Nuno FJ, Fernandez Alvarez R, Perez-Gonzalez F, Asensi V. Diabetes mellitus and bacteraemia: a comparative study between diabetic and non-diabetic patients. *Eur J Med*. 1992;1(5):281–287.
16. Graham BB, Keniston A, Gajic O, Trillo Alvarez CA, Medvedev S, Douglas IS. Diabetes mellitus does not adversely affect outcomes from critical illness. *Crit Care Med*. 2010;38(1):16–24. https://doi.org/10.1097/CCM.0b013e3181b9eaa5.
17. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Med*. 2017;43(3):304–377. https://doi.org/10.1007/s00134-017-4683-6.
18. Brusselaers N, Vogelaers D, Blot S. The rising problem of antimicrobial resistance in the intensive care unit. *Ann Intensive Care*. 2011;1:47. https://dx.doi.org/10.1186%2F2110-5820-1-47.
19. Pitout JD. Infections with extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: changing epidemiology and drug treatment choices. *Drugs*. 2010;70(3):313−333. https://doi.org/10.2165/11533040-000000000-00000.
20. Zhanel GG, DeCorby M, Ling N, et al. Antimicrobial-resistant pathogens in intensive care units in Canada: results of the Canadian National Intensive Care Unit (CAN-ICU) Study, 2005–2006. *Antimicrob Agents Chemother*. 2008;52(4):1430–1437. https://doi.org/10.1128/AAC.01538-07.
21. Knathe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Transferable resistance of cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella Pneumoniae* and *Serratia marcenscens*. *Infection*. 1983;11(6):315–317. https://doi.org/10.1007/bf01641355.

22 Sharma M, Pathak S, Srivastava P. Prevalence and antibiogram of extended spectrum β-lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and *Klebsiella* spp. *J Clin Diagn Res*. 2013;7(10):2173–2177. https://doi.org/10.7860/JCDR/2013/6460.3462.

23 An D, Rodrigues B. Role of changes in cardiac metabolism in development of diabetic cardiomyopathy. *Am J Physiol Heart Circ Physiol*. 2006;291(4):H1489–H1506. https://doi.org/10.1152/ajpheart.00278.2006.

24 Ratnadeep B, Oudit GY, Wang X, et al. Type 1 diabetic cardiomyopathy in the Akita (Ins2$^{WT/C96Y}$) mouse model is characterized by lipotoxicity and diastolic dysfunction with preserved systolic function. *Am J Physiol Heart Circ Physiol*. 2009;297(6):H2096–H2108. https://doi.org/10.1152/ajpheart.00452.2009.

25 Geerlings SE. Urinary tract infections in patients with diabetes mellitus: epidemiology, pathogenesis and treatment. Int J Antimicrob Agents. 2008;31(1):S54–S57. https://doi.org/10.1016/j.ijantimicag.2007.07.042.