routine diagnostic TB laboratories would employ this standard operating procedure for the isolation of *M. tuberculosis*, but data from the present study indicate that it is not optimal for the isolation of NTM from CF sputum.

Recovery of mycobacteria using NaOH was poor in comparison to those methods utilising oxalic acid. This may be due to the fact that NTM organisms are more susceptible to killing by NaOH, in comparison to *M. tuberculosis* organisms. Furthermore, this NaOH method proved less able to reduce the background Gram-negative co-flora than were the other two oxalic acid methods tested, as adult CF sputum has been shown to contain high numbers of Gram-negative organisms ($10^8$–$10^9$ cfu/mL).[8] Both oxalic acid methods showed greater sensitivity for the isolation of *M. abscessus*, and decontamination methods using oxalic acid demonstrated greater ability to remove the Gram-negative co-flora than did use of NaOH alone.

In conclusion, this study demonstrated a low isolation rate of NTM organisms in the adult CF patient population examined, but was not able to link any predisposing factors, such as longevity or environmental exposure and use of antibiotics, with infection/colonisation by NTM. Overall, the 0.5% NALC/2% NaOH/5% oxalic acid decontamination method proved to be the optimal method for the decontamination of CF sputum. Clearly, the establishment of a standardised decontamination method for use with CF sputa would provide more accurate results for NTM isolation, thus enhancing the understanding of their role as opportunistic pathogens in cystic fibrosis.

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# Extreme hyperglycaemia and hypokalaemia in a 96-year-old diabetic patient: case report and discussion of management issues


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Extreme hyperglycaemia is a common and serious diabetic emergency that may progress to a hyperglycaemic hyperosmolar state (HHS), which is a diabetic crisis occurring predominantly in elderly type 2 diabetics and characterised by dehydration, severe hyperglycaemia and slight or no ketosis. Fluid replacement, insulin therapy and potassium repletion are the main principles of treatment in hyperglycaemic crises.[1-3] However, the decision about when and how to initiate these measures needs to be individualised as there are many pitfalls along the route to successful treatment. Here, methods are reported for managing an extremely old type 2 diabetic patient who survived extreme hyperglycaemia and hypokalaemic.

A 96-year-old woman was referred to the emergency room following a six-day history of nausea, vomiting and fatigue, and confusion for one day. She had been diagnosed with type 2 diabetes some 17 years previously and had been using oral hypoglycaemic agents such as metformin, α-glucosidase inhibitors and sulfonylureas, and, more recently, glipizide.


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**Table 1.** Laboratory results.

| Date | Time | Glu (mmol/L) | Na+ (mmol/L) | Corrected Serum $Na^+$ (mmol/L) | $K^+$ (mmol/L) | Cr (μmol/L) | Bun (mmol/L) | WBC ($x10^9$/L) | N (%) | Hb (g/L) | Hct | Serum osmolarity (mOsm/kg $H_2O$) Total | Effective |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15/2 | 14:00 | 27.9 | 140.0 | 146.4 | 3.14 | 107 | 12.4 | | | | | 320.3 | 307.9 |
| | 18:00 | 17.9 | 145.4 | 148.9 | 3.17 | 92 | 12.3 | 14.4 | 84.5 | 157 | 0.434 | 321.0 | 308.7 |
| | 21:00 | 14.1 | 146.1 | 148.5 | 3.71 | | | | | | | | 306.3 |
| 16/2 | 6:00 | 14.4 | 145.5 | 148.0 | 3.31 | 89 | 9.3 | 13.2 | 83.6 | 139 | 0.414 | 314.4 | 305.1 |
| | 14:00 | 7.2 | 144.9 | 145.4 | 3.84 | 88 | 7.8 | | | | | 304.8 | 297.0 |
| 17/2 | 6:00 | 9.7 | 137.7 | 138.9 | 3.64 | 70 | 5.8 | | | | | 290.9 | 285.1 |
| 18/2 | 6:00 | 8.6 | 135.3 | 136.2 | 3.51 | 79 | 6.7 | 12.7 | 72.2 | 135 | 0.391 | 285.9 | 279.2 |
| 19/2 | 6:00 | 7.5 | 133.6 | 134.1 | 3.44 | | | 10.9 | 74 | 127 | 0.363 | | 274.7 |
| 20/2 | 6:00 | 6.5 | 136.7 | 137.0 | 3.83 | 73 | 5 | | | | | 284.9 | 279.9 |

Her fasting plasma glucose levels had been 8–10 mmol/L, and her two-hour post-prandial glucose levels had been 15–16 mmol/L.

Six days before admission, the patient suffered from dizziness, fatigue, nausea and vomiting. She had no abdominal pain and no diarrhoea. Four days before admission, her fasting plasma glucose level was 11 mmol/L and her serum electrolyte values were normal. However, she had eaten very little food and drank about 250 mL of water per day. Her daily urine output was approximately 1000 mL. One day before admission she began to become confused.

On admission, her temperature was 36.8°C, heart rate was 120 beats/min with atrial fibrillation, respiration was 20 breaths per minute and blood pressure was 150/90 mmHg. Her plasma glucose concentration was 27.9 mmol/L, serum sodium level was 140 mmol/L, potassium 3.14 mmol/L, blood urea nitrogen 12.4 mmol/L (Table 1), urinary ketone was negative and blood pH was 7.46. She was transferred to the intensive care unit (ICU).

The patient had suffered hypertension for about 40 years and was treated with amlodipine (5 mg per day). Atrial fibrillation had been diagnosed six years previously and she had a colostomy as a result of surgery for rectal cancer.

On physical examination, the patient was confused, with poor tissue turgor, dry buccal mucosal membranes and soft, sunken eyes. No enlarged lymph nodes were palpable and there was no bulging of the jugular vein. There were no rales in the lungs and the patient's heart rate was 120 beats per minute, with atrial fibrillation but no audible murmur. The abdomen was soft and not tender, with no signs of organomegaly. There was no peripheral oedema. The musculoskeletal system was normal and the Babinski signs were negative.

As the patient's serum potassium concentration was low (3.14 mmol/L), insulin therapy was not initiated, but 500 mL normal saline (NS) together with 1.5 g potassium were given intravenously. A bedside glucose meter was used to monitor the patient's capillary glucose level hourly (Table 2). Three hours later, the patient's glucose level fell to 14.1 mmol/L (Table 1). In order to halt the decreasing plasma glucose, insulin was still not given, although at this time her serum potassium level was 3.71 mmol/L. Thus, for the following nine hours, 1000 mL NS together with 1.5 g potassium were infused.

Approximately 12 hours after admission, the patient's mental awareness began to improve, urine output increased to 1560 mL, and her heart rate and blood pressure stabilised. Her plasma glucose level was 14.4 mmol/L, serum sodium 145.5 mmol/L and potassium 3.31 mmol/L.

Over the next 24 hours, the patient was given 1000 mL NS and 2000 mL 5% glucose solution (GS) in conjunction with 6 g potassium intravenously. As her plasma potassium was stable and >3.3 mmol/L, three units per hour of regular insulin were administered. Urine output was maintained at approximately 2200 mL over 24 hours. Then, 200 mL Glucerna and 200 mL water were given for 6 h, respectively, through a nasogastric tube, and intravenous fluid replacement gradually was decreased.

With these measures, the patient's serum total and effective osmolarity gradually decreased (Table 1), with a consequent progressive improvement in mental state. She recovered full consciousness 24 hours after admission.

When the patient could eat and had returned to a near-normoglycaemic state, a transition from intravenous to subcutaneous insulin therapy was undertaken, with the dose adjusted according to capillary blood glucose results. One week after admission, the patient's fasting plasma glucose level was stable at 6–8 mmol/L, so she was switched from insulin to gliquidone and the patient was discharged shortly afterwards.

The patient has been followed-up for almost 12 months and shows generally good glycaemic control, with fasting

**Table 2.** Capillary glucose level during the first 24 hours.

| | 15/2/2007 | | | | 16/2/2007 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14:00 | 18:00 | 20:00 | 22:00 | 0:00 | 2:00 | 6:00 | 8:00 | 10:00 | 12:00 | 14:00 |
| Glucose (mmol/L) | 27.9 | 17.9 | 14.7 | 14.2 | 14.7 | 15.0 | 14.4 | 14.9 | 10.5 | 11.5 | 7.2 |

glucose concentration of approximately 8 mmol/L and two-hour post-prandial glucose concentration of approximately 10 mmol/L.

Extreme hyperglycaemia is a common and serious diabetic emergency that may progress to HHS if not treated properly. Laboratory findings in patients with HHS include marked elevations in both blood glucose and serum effective osmolarity.

Although the patient reported here cannot be diagnosed as HHS according to serum effective osmolarity, her serum osmolarity level was above the normal range. In addition, at the age of 96 years, she had hyperglycaemia and coexisting atrial fibrillation and hypertension, and had a history of rectal cancer.

Hyperglycaemia is a volume-depleted state, with water deficits of approximately 9 L. This may be attributed to the more gradual onset and longer duration of metabolic decompensation and partially to the fact that patients have an impaired fluid intake.[4]

The patient had been sick for six days prior to admission. Her biochemical parameters demonstrated elevated blood levels of creatinine (Cr) and urea nitrogen (Bun), as well as an increased haematocrit (Hct), all of which are indicative of dehydration.

Hyperglycaemia is the most common cause of translocational hyponatraemia. The principal mechanism of glucose-induced hyponatraemia is movement of water from the intracellular fluid compartment to the extracellular fluid compartment, due to restriction of glucose to the extracellular space. An increase of 5.6 mmol/L (100 mg/dL) in serum glucose concentration decreases serum sodium by approximately 1.6 mmol/L, resulting in a rise in serum osmolarity of approximately 2.0 mOsm/kg $H_2O$.[5]

Using a standard correction factor of 1.6, on admission the patient's corrected serum sodium was 146.3 mmol/L. Some studies report that the physiological decrease in sodium concentration is considerably greater than the standard correction factor of 1.6, especially when glucose concentration is greater than 22.4 mmol/L (400 mg/dL). Thus, a correction factor of 2.4 is a better overall estimate of this association when the glucose concentration is >22.4 mmol/L (400 mg/dL).[6]

For patients in diabetic crisis, fluid replacement is one of the mainstays of treatment, and should be started immediately with normal saline.[7] In addition, for those patients who have neurological symptoms, supplying fluid through a nasogastric tube is advisable. The patient reported here was given 1600 mL/day of fluid through a nasogastric tube, thus ensuring adequate fluid replacement without inducing heart failure.

Although not recommended and even discouraged for fear of acidaemia, it is possible to treat hyperglycaemic type 2 diabetic patients with fluids alone.[3] Fluid replacement by itself can greatly decrease serum glucose concentration at a rate of 4.4–11.1 mmol/L per hour (80–200 mg/dL per hour) through dilution (24–36% decline), improve renal perfusion and reduce the level of counter-regulatory hormones.[8–10] The changes in plasma glucose and serum osmolarity seen in the current patient after fluid supplement alone supports this concept.

Administration of insulin may lead to the intracellular transport of glucose, with a resultant fluid shift from the intravascular to intracellular space and hypotension, if adequate hydration is not first achieved. Patients with hyperosmolarity who have an adequate urine output and blood pressure on hospital admission may become oliguric and hypotensive once the glucosuric osmotic load dissipates, especially when plasma glucose level is lowered to 13.8–19.4 mmol/L (250–350 mg/dL) by insulin.[3]

Hyperglycaemia causes an osmotic diuresis with dehydration and loss of electrolytes, particularly the cations $Na^+$, $K^+$ and $Mg^{2+}$. Hyperglycaemia may be associated with profound total body potassium depletion, in the order of 3–15 mmol/kg of body weight.[11] However, plasma potassium concentration is typically normal or elevated at the time of presentation.

Several mechanisms could explain this phenomenon. Water can remove potassium out of the cells, as, during acidosis, $H^+$ moves into cells in exchange for $K^+$. Loss of intracellular phosphate leads to intracellular $K^+$ to maintain electrical neutrality. Insulin deficiency impairs movement of potassium into cells. Insulin deficiency leads to breakdown of glycogen and protein, with the movement of associated potassium out of the cell.[12]

Although the patient's serum potassium was moderately low (3.17 mmol/L), this finding alerted clinicians to the potential severity of total body potassium depletion due to osmotic diuresis. However, the use of insulin may mediate the intracellular transport of potassium, and the plasma potassium concentration will invariably fall. Thus, when serum potassium level is <3.3 mmol/L, insulin therapy should be delayed until potassium concentration is restored to a level >3.3 mmol/L in order to avoid arrhythmia, cardiac arrest and respiratory muscle weakness.[1,11]

In the present case, insulin therapy was not initiated during the first 12 hours. Fortunately, the patient's glucose concentration dropped from 27.9 mmol/L to 14.4 mmol/L, and her effective plasma osmolarity dropped gradually.

It is recommended that the rate of serum osmolarity reduction should not exceed 3 mOsm/kg $H_2O$ per hour in order to avoid brain oedema. The simplest way to control the drop in serum osmolarity is to control the speed at which plasma glucose changes. On the other hand, plasma glucose concentration should be maintained at 13.9–16.7 mmol/L until the patient's mental state is restored.[13]

In summary, despite advances in the field, hyperglycaemia continues to carry a significant risk of mortality, especially in very old patients. An important point highlighted by the case presented here is that successful treatment of hyperglycaemia relies on not initiating insulin therapy until potassium had reached a predetermined value. Clearly, it is important to understand the pathophysiology of the disease and to interpret each laboratory result correctly. Treatment should then be given on an individual basis, taking into account current guidelines and recommendations.

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# Difficult-to-identify bacteria: how use of 16S rDNA PCR and gene sequencing can help

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Universal or 'broad range' eubacterial polymerase chain reaction (PCR) was performed on a collection of 70 phenotypically difficult-to-identify bacterial isolates, including 31 atypical mycobacterial isolates, originating from the routine service of an NHS clinical microbiology laboratory in the UK. 16S rDNA PCR was performed using two sets of universal primers to generate a composite amplicon of 1068 bp, which was sequenced to obtain each isolate's identity. In most cases, sequence analysis was able to identify the isolates examined with relative ease, with the respiratory section (atypical mycobacteria and cystic fibrosis) and blood culture work as the main beneficiaries of adoption of molecular identification methods. When the use of molecular identification methods is justified, employment of partial 16S rDNA PCR and sequencing provides a valuable and reliable method for the identification of bacteria that have proved difficult to identify by phenotypic techniques.

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Correct identification of bacterial organisms in clinical microbiology is an important laboratory function. Such organisms may be presented for identification from sporadic clinical cases, from outbreaks or for epidemiological or surveillance purposes. Such identification, to date, has relied largely on phenotypic schema, including initial examination of colonial morphology and Gram stain, which today is usually followed by some form of semi-automated identification scheme, generally based on biochemical differentials such as the API identification schemes, the BBL Crystal Scheme or Vitek 2.

However, one problem with such systems is that their databases are incomplete for some organisms of clinical significance, thereby creating identification anomalies in trying to identify correctly such cultures, even to the genus level. Molecular methodologies offer an alternative laboratory mechanism for the identification of such organisms, particularly the employment of 16S rDNA PCR and sequencing techniques. Over the past decade, particularly with the adoption of PCR in biomedical science, such techniques have been employed increasingly in routine laboratory diagnostics.

Given that 16S rDNA PCR and automated sequencing has become a routine tool in many specialist and reference microbiology laboratories, little more can be added to the description of the method. However, what is of interest is how such techniques can aid the busy service laboratory, and what value such techniques add. Hence, the aim of this study is to discover which organisms are being forwarded for molecular analysis from the phenotypic section, due to difficulties in identification.

Viable culturable bacterial isolates ($n$=70; 31 atypical mycobacterial isolates and 39 difficult-to-identify isolates from various microbiology laboratory sections) were examined in order to obtain an identification by 16S rDNA PCR and direct automated sequencing, in accordance with a standard protocol.[1] All isolates originated from the routine microbiology service of a large UK hospital trust, which processes approximately 160,000 clinical specimens per annum and has specialist centres for adult cystic fibrosis (CF) and haematological malignancy.

Atypical mycobacterial isolates were examined from the archive of such isolates stored in the Northern Ireland Mycobacterium Reference Laboratory, situated within the same clinical microbiology department. The criteria for forwarding such isolates for molecular analysis included (i) poor (or no) discrimination of identification of the isolate using combinations of phenotypic and conventional identification assays, as performed by experienced biomedical scientists, and (ii) the likelihood of the isolate being of potential clinical significance by the consultant medical microbiologist. All isolates requiring such molecular analysis were processed for 16S rDNA PCR and direct automated sequencing, as described previously.[1]

A total of 70 isolates were obtained for 16S rDNA PCR and sequencing purposes, as detailed in Tables 1 and 2. Polymerase chain reaction amplifications on high-quality genomic DNA preparations of these isolates generated