appropriate routine genotypic testing of *E. faecium* clinical isolates is recommended, particularly those from sterile sites, to determine the presence of vancomycin resistance genes.

Should we worry about the colonisation of VVE since the routine non-molecular VRE screening methods do not reliably identify VVE and did not detect the index case summarised here? A large hospital outbreak due to VVE has been previously described in Ontario⁹ and Denmark,² thus highlighting that VVE is able to spread within the community and health care facilities. Further research is required to determine the sensitivity of various selective agars with and without vancomycin in detecting VVE and whether this strategy in combination with molecular testing is cost-effective.

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Genomic sequencing of hypervirulent *Klebsiella pneumoniae* with novel patterns of virulence and global epidemiological linkage

Sir,

Klebsiella pneumoniae is an increasingly important cause of infections in humans due to its capability to acquire a range of virulence factors and antibiotic resistance genes.^{1,2} Many infections occur in hospitalised patients with impaired host defences usually as a consequence of immunodeficiency or chronic comorbidity.^{1,2}

Hypervirulent *K. pneumoniae* have also emerged, particularly in Asia, and can cause invasive infections in healthy hosts.^{1,2} Clinical disease is typically characterised by primary hepatic abscesses, with complications of endophthalmitis, meningitis, and further metastatic abscesses.^{1,2} Hypervirulent variants often demonstrate a hypermucoid phenotype, as determined by the 'string test'.^{1,2}

Key genotypic factors associated with increased virulence include: mucoid phenotype genes *rmpA/A2*; iron acquisition systems salmochelin, aerobactin, yersiniabactin, and the genotoxin colibactin. These virulence factors are carried by mobile genetic elements. *Klebsiella pneumoniae* isolates usually harbour a subset, or none, of the genes encoding for these virulence factors. Strains carrying a full hand of virulence factors have rarely been reported to cause prostatic abscesses,^{1,2} and usually only in the context of metastatic disease with additional risk factors such as diabetes mellitus.¹ Men who have sex with men (MSM) have not been reported to be at increased risk.

A previously well, 23-year-old man of Chinese ancestry, presented to a hospital in Melbourne, Australia, with three days of fevers, dysuria, and purulent urethral exudate culminating in urinary retention on the day of presentation. The patient had been in a stable sexually active relationship with a male partner for the previous 6 months. The patient practised only receptive anal intercourse without regular condom use. Three months prior to presentation, he had travelled to Shanghai, China to visit friends and relatives. He reported no sexual contact while overseas.

On presentation, the patient was febrile at 38.6°C, tachycardic at 136 beats/min, tachypnoeic at 24 breaths/min, and had a blood pressure of 132/83 mmHg. Examination noted purulent urethral exudate, lower abdominal pain without peritonism, and a tender prostate. The remainder of the examination was unremarkable.

The peripheral leukocyte count was $9.2 \times 10^9/L$ (normal $4.0\text{--}12.0 \times 10^9/L$) and a C reactive protein (CRP) was 69.3 mg/L (normal <5.0 mg/L). Renal function was normal, and liver function testing revealed a mild transaminitis with an

elevated ALT of 57 U/L (normal 5–40 U/L) and AST of 62 U/L (normal <35 U/L). HIV and syphilis serology, and urinary PCR for chlamydia and gonorrhoea were negative.

An indwelling catheter was inserted. Computed tomography scanning revealed a peripherally enhancing 24×30×27 mm collection within the prostate, consistent with a prostatic abscess. There was thickening and fat-stranding of the bladder and inflammation of the seminal vesicles (Fig. 1). The remaining intra-abdominal organs, including liver, were unremarkable.

Urine microscopy showed 1422×10^6 /L leukocytes. Urine culture grew *K. pneumoniae* with $>10 \times 10^8$ CFU/L. *Klebsiella pneumoniae* was also recovered from a urethral swab. Susceptibility testing noted only ampicillin resistance. The phenotypic string test was positive. Blood cultures prior to antimicrobial therapy were negative.

The patient received intravenous cephazolin for 5 days, followed by oral ciprofloxacin. Surgical drainage of the prostatic abscess was considered but conservative management preferred due to operative risks of incontinence and erectile dysfunction in a sexually active young man. The indwelling catheter remained *in situ* for 4 weeks and the patient completed a 6 week course of oral ciprofloxacin. Interval prostatic ultrasound after 2 weeks and 4 weeks showed improvement of the abscess. The indwelling catheter was removed at 4 weeks with return to normal voiding. CRP was 3.4 mg/L and liver function had normalised at completion of treatment, and the patient has remained well on follow-up.

DNA extraction from single colonies was performed using the MagJET Genomic DNA purification kit (ThermoFisher Scientific, Australia). Genome sequencing of the isolate (AUSMDU00016625) on the Illumina Nextseq 550 (Illumina, USA) provided 6,109,938 reads with $175 \times$ sequence depth. These were assembled with the genome assembly program SPAdes v3.12.0 into 55 separate contiguous DNA segments (contigs). Phylogenetic inference to determine relatedness to other isolates was performed with the program Snippy v4, and visual representation with a maximum likelihood tree was created in IQtree (Fig. 2).

Fifty-one of the 55 assembled contigs were found to correspond to chromosomal DNA (5.1 Mb). The four remaining contigs corresponded to three separate plasmids: one small plasmid of 5 kb and two large plasmids (210 kb and 100 kb). We profiled AUSMDU00016625 for virulence factors, surface antigens for *in silico* typing, and antimicrobial resistance genes using *Klebsiella* genome assembly databases Kleborate and Kaptive.^{3–7}

The isolate was sequence type 592 (ST592) and capsule type K57. There was no acquisition of antimicrobial resistance genes, with only the intrinsic ampicillin resistant genes *ampH* and *bla_{SHV}* present. All five major virulence factors were present: yersiniabactin, colibactin, aerobactin, salmochelin, and the *rmpA* hypermucoid phenotype gene (Fig. 2).

There were 11 other ST592 isolates in the Bacterial Isolate Genome Sequencing Database (BIGSdb) available for comparative genomic analysis, with all of the human clinical isolates originating from Asia. ST592 are single locus variants (SLV) of ST34 and are closely related. Phylogenetic analysis clustered our isolate, AUSMDU00016625, with other ST592/34 isolates from multiple different countries. Other ST592 (or ST592-SLV) isolates carried only three of the five virulence factors, aerobactin, salmochelin, and the *rmpA* hypermucoid phenotype gene, and had variable antimicrobial resistance (Fig. 2).

The first large plasmid (210 kb) carried *rmpA* and the genes encoding for aerobactin and salmochelin. Sequencing yielded 100% coverage of the plasmid within a single contig. The plasmid was a 99.54% match to a virulence plasmid carried on isolate WCHKP13F2, a hypervirulent, multidrug resistant, ST36 isolate from Sichuan, China. This ST36 isolate itself is phylogenetically very distinct to ST592 isolate AUSMDU00016625 (Fig. 2).

Yersiniabactin was found to be encoded on a mobile integrative conjugative element (ICE) within the chromosomal DNA of AUSMDU00016625, which is also carried on an ICE by the same WCHKP13F2 isolate from China.

The colibactin gene was found within a single contig in the chromosomal DNA, and did not correspond to any mobile genetic elements. It is known to be carried by many different

Fig. 1 (A) Contrast enhanced computer tomography scanning through the prostate, demonstrating a loculated collection surrounding the prostatic urethra with peripheral contrast enhancement and central hypoattenuation (red arrow), consistent with a prostate abscess. (B) Contrast enhanced computer tomography scanning through the bladder and seminal vesicles, demonstrating inflammation of the bladder wall consistent with cystitis (red arrow), and cystic inflammation of the seminal vesicles (yellow arrow) consistent with involvement with infection from the prostatitis.

Fig. 2 Maximum likelihood tree of core genome phylogenetic relationship of AUSMDU00016625 to 31 other *K. pneumoniae* isolates of sequence type (ST) 592, ST66, ST36 ST34 and ST228. Highlighted region in grey on the tree is the clonal cluster of ST592/ST34. Presence of antimicrobial resistance genes are colored blue circles and virulence factors in red circles. The AUSMDU00016625 isolate is highlighted with a red box, and the WCHKP13F2 isolate from Sichuan, China with a black box. NA, not available.

sequence types, but notably is also present in WCHKP13F2 isolate.

The second plasmid (100 kb) was a close genetic match to a non-clinical, laboratory reference ST66 isolate (Kp52.145), and does not encode any known virulence factors.

This patient may have had an ascending urethral infection complicated by prostatic abscess. MSM are at increased risk for urinary tract infections compared to other males; however, the increased risk is thought to be related to insertive anal intercourse,⁸ which this patient did not report. An alternative hypothesis is of rectal colonisation by hypervirulent *K. pneumoniae* followed by inoculation into the prostate during receptive anal intercourse. Supportive lines of evidence include the absence of bacteraemia or metastatic infection to suggest infection seeded from elsewhere. Gastrointestinal carriage with multidrug resistant *Enterobacteriaceae* acquired during travel is well described⁹ and can predispose to invasive *K. pneumoniae* infections,¹⁰ and prostatic abscesses can be a complication of transrectal prostate biopsy.¹¹

Transmission of mobile genetic elements between strains of *Klebsiella pneumoniae* carrying both determinants of antimicrobial resistance and/or virulence have been previously well described.¹² The strain AUSMDU00016625 has a distinct genetic background to WCHKP13F2, but has acquired its mobile genetic elements (plasmids and ICE) harbouring key virulence factors. This raises the possibility of gastrointestinal colonisation with different strains of *K. pneumoniae* (including the WCHKP13F2 strain acquired in China), transmission of virulence factors between strains, and subsequent clinical infection with the recombinant hypervirulent isolate.

This report presents a novel clinical scenario of a primary prostatic abscess due to a hypervirulent

K. pneumoniae, without evidence of metastatic complication or bacteraemia, in a previously healthy young male. We hypothesise gastrointestinal colonisation and recombination of strains following recent travel to China and subsequent urinary tract infection, possibly related to unprotected receptive anal intercourse. This is also the first report of a *K. pneumoniae* ST592 with a hypervirulent phenotype and harbouring all five key virulence factors. The comparative genomic analysis reaffirms the global concern of transmissible mobile genetic elements in our modern era of globalisation, and is an important reminder about transmissible virulence, and not just transmissible antimicrobial resistance.

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SMOFlipid causing spuriously high serum total bilirubin in an extremely premature neonate

Sir,
Neonatal jaundice caused by unconjugated bilirubin is a common problem in neonates.¹ Bilirubin is a product of heme breakdown through a cascade of enzyme-catalysed reactions in the reticuloendothelial system.² *In utero* the fetus maintains a high haemoglobin concentration to effectively extract oxygen from maternal blood which has relatively low oxygen tension. Once *ex utero* this requirement falls significantly with the availability of abundant oxygen through breathing ambient air reducing the need for elevated haemoglobin. This, coupled with the shorter fetal erythrocyte lifespan, produces an excess load of haemoglobin, which is metabolised into bilirubin in the immediate post-natal period.

However, the high bilirubin load can easily overwhelm the immature excretory system, predisposing neonates to developing jaundice in the first week of life. Unconjugated, free bilirubin can cross the immature neonatal blood brain barrier, causing selective damage of the central nervous system.^{3–5}

With acute bilirubin toxicity in neonates, there is a progression of symptomatology beginning with lethargy and decreased feeding, and progressing to variable changes in tone, high-pitched cry, seizures, and even death.

Here, we describe a case of an extremely premature female baby with a progressive rise in serum bilirubin, which could not be accounted for by clinical progression and whereby the serum bilirubin values measured by two different laboratory methods were vastly divergent. This case highlights the limitations of standard laboratory methods in measuring bilirubin for extremely premature infants and the importance of clinical correlation and communication between disciplines in reaching a definite diagnosis to guide management options.

The patient was an extremely preterm female baby of about 24 weeks of gestation. Her birthweight was less than 500 g. She was delivered via emergency caesarean section due to worsening maternal pre-eclampsia. After initial resuscitation efforts, the baby was admitted into the neonatal intensive care unit (NICU). In the NICU, she received total parenteral nutrition, which contained 1–2 g/kg/day of lipids (SMOFlipid, an emulsion of fish oil, medium chain fatty acids, olive oil, soybean oil). SMOFlipid is given as a source of calories and essential fatty acids for parenteral nutrition to premature babies by day 1–2 of life because they cannot meet their nutritional needs using the enteral route in early days of life.

The patient's daily serum total bilirubin concentrations were monitored with heel prick blood sampling and direct spectrophotometry bilirubinometer (Unistat Bilirubinometer; Reichert Technologies, USA). At 24 hours of life, her bilirubin was 86 µmol/L (Fig. 1); hence, phototherapy was started according to hour-specific phototherapy threshold (80 µmol/L) for extremely low birth weight babies.¹ There were no other risk factors for jaundice as she was not glucose-6-phosphate dehydrogenase deficient and there was no evidence of ABO blood group incompatibility.

Eight hours after starting phototherapy at 30 hours of life, her bilirubin concentration was 196 µmol/L. Her phototherapy was intensified to 6 phototherapy units with exposure of her entire body surfaces. At 43 hours of life, despite being on intensive phototherapy, her serum bilirubin increased further to 220 µmol/L, exceeding the recommended level of exchange transfusion (170 µmol/L).¹ To investigate the worsening hyperbilirubinaemia, a complete blood count and liver function test were performed. Simultaneously, another heel prick blood sample was collected for testing on the bilirubinometer.

Her bilirubin measured by bilirubinometer was elevated at 293 µmol/L. However, the corresponding bilirubin from the liver panel was abnormally low at 15 µmol/L (term day 3 reference intervals 75–120 µmol/L; measured using AU58000, Beckman Coulter, USA). Aspartate aminotransferase (182 U/L; reference 30–160 U/L) and lactate dehydrogenase (5915 U/L; reference: 590–2300 U/L) were elevated. Complete blood count showed normochromic, normocytic anaemia (haemoglobin 100 g/L; reference 120–180 g/L), and high reticulocyte count ($132 \times 10^9/L$). Presence of anaemia, high lactate dehydrogenase measurement, and high reticulocyte count indicated haemolysis. Her white cell count and C-reactive protein, total protein, albumin, alanine aminotransferase, and renal function were normal.

Treatment for hyperbilirubinaemia, either intensive phototherapy or exchange transfusion, should always be