

Fatal sepsis in a pregnant woman with pyelonephritis caused by *Escherichia coli* bearing Dr and P adhesins: diagnosis based on postmortem strain genotyping

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Case report

A 23-year-old Polish woman in her first pregnancy was admitted to the hospital at 33 weeks of gestation, complaining of uterine contractions every 5 minutes and a prenatal history of vomiting and constipation. On examination her general condition was good, her temperature was 36.6°C, with a heart rate of 76/minute, and blood pressure of 120/80 mmHg. There were no cutaneous eruptions or focal inflammatory lesions. The fetal lie was longitudinal and the fetal heart rate was 140/minute. Fetal movements were palpable during the examination. The cervix was 50% effaced and 2-cm dilated, and there was no rupture of amniotic membranes. Her provisional diagnosis was preterm labour.

The wet mount was positive for a high level of leucocytes and bacteria, and was negative for clue cells. Her urine pH was 6.0, with specific gravity 1020, protein 1.0 g/l, ketones 10 mmol/l, leucocyturia 500/ml, and erythrocytes 250/ml. There were a large number of bacteria in the microscopic field. The urine sample was sent for culture. The patient was given intravenous phenotholol and verapamil for tocolysis, Scopolan (hyoscine butylbromide) 10 mg three times a day, No-Spa (drotaverine hydrochloride) 40 mg twice a day, and betamethasone (2 × 12 mg) for fetal lung maturity. The contractions resolved over the first 2 days of hospitalization. By the third day, at

03:30 hours, her condition deteriorated. Her temperature rose to 38.5°C, and her heart rate and blood pressure were recorded as 88/minute and 110/70 mmHg, respectively. A dose of oral paracetamol (500 mg) was administered, and intravenous Augmentin (1000 mg of amoxicillin and potassium clavulanate, equivalent to 200 mg clavulanic acid) was given every 8 hours. Her temperature rose further to 39.5°C at 04:00 hours, and by 04:15 hours there was a rapid deterioration in her general condition. Her speech was slurred and limited, but she was responsive to painful stimuli. Her heart rate rose to 120/minute, her systolic blood pressure was only 60 mmHg, and she was emitting foamy exudate from her mouth. A diagnosis of massive pulmonary oedema as a result of acute circulatory and respiratory insufficiency was made, probably as a result of urosepsis.

Cardiopulmonary resuscitation was initiated, but this was ineffective. A peri-mortem caesarean section was performed to save the baby's life. The patient died at 05:03 hours. A postmortem urine culture grew *Escherichia coli* that was susceptible to all antibiotics tested, except ampicillin and piperacillin. Analysis of previously unavailable pregnancy medical documentation revealed the use of amoxicillin with clavulanic acid and nitrofurantoin for ambulatory treatment of a urinary tract infection.

An autopsy was performed 24 hours postmortem, and showed hydronephrosis and acute pyelonephritis, with

abscesses in the left kidney, pulmonary congestion and oedema, cerebral congestion and oedema, with signs of transtentorial herniation. Histopathological examination showed tubulointerstitial nephritis with microabscesses and chronic suppurative pyelitis in the kidney, an acute septic reaction with large numbers of neutrophils in congested sinuses of the spleen and leukocytosis in the sinuses of the liver. There was a focal contraction band necrosis of the heart, pulmonary microvascular embolisms from syncytiotrophoblasts and megakaryocytes and oedema, and focal intra-alveolar haemorrhages in the lungs. The primary cause of death was judged to be *E. coli* sepsis as a complication of pyelonephritis. Postmortem cultures of samples from her blood, kidney, lung, brain and spleen grew a strain of *E. coli* that was resistant only to ampicillin and piperacillin. Polymerase chain reaction (PCR) assays for the detection of specific virulence factors characteristic for uropathogenic *E. coli* were performed as previously described,^{1,2} using six sets of primers targeting the following virulence factors: type-P fimbriae (*papC*), type-S fimbriae (*sfa*), cytotoxic necrotizing factor 1 (*cnf1*), uropathogenic specific protein gene (*usp*), type-1 fimbriae (*fim*) and α -haemolysin (*hlyA*). In a separate PCR assay the presence of Dr fimbriae (*draC*) was tested. All tested virulence factors were detected in all *E. coli* isolated during the autopsy. The *E. coli* genotyping was performed for all isolates tested using PCR melting-profile (MP) analysis and restriction enzyme analysis pulse-field gel electrophoresis (REA-PFGE) according to a previously described procedure.^{1,2} The results revealed 100% similarity between band patterns for both methods (Figure 1), indicating that all of

the isolates were unambiguously derived from a common progenitor *E. coli*. These results confirmed that the death was attributable to *E. coli* sepsis as a complication of acute pyelonephritis/chronic pyelitis.

Discussion

We present the first fatal case of gestational interstitial tubulonephritis and chronic pyelitis caused by Dr⁺ *E. coli* in a young pregnant woman. Postmortem examination showed that she died from Dr⁺ *E. coli* septicaemia of renal origin. Pyelonephritis in pregnancy is associated with multiple complications, including fetal growth restriction, preterm labour, cerebral palsy and septicaemia, although the underlying mechanisms are poorly understood.³

The establishment of urinary tract infection (UTI) outside pregnancy requires the adherence of *E. coli* to epithelial receptors, which facilitate the ascending colonisation of the urogenital tract.⁴ To date, UTI in pregnancy is explained by obstruction of urine outflow by the gravid uterus, with little attention paid to the pregnancy-related alterations in the immune system, and the receptor-adherence etiology of the ascending UTI. Our prior analysis of *E. coli* virulence factors in gestational pyelonephritis showed a predominance of *E. coli* bearing Dr adhesins (Dr⁺ *E. coli*): 27% of UTI in the first, 26% in the second and 39% in the third trimesters.⁵ We propose that Dr⁺ *E. coli* can invade the renal interstitium, escape killing by leukocytes and persist within epithelial cells. Using an experimental rat model, Dr⁺ *E. coli* was not only lethal during pregnancy, but resulted in chronic interstitial nephritis.^{6–9} We refer to the

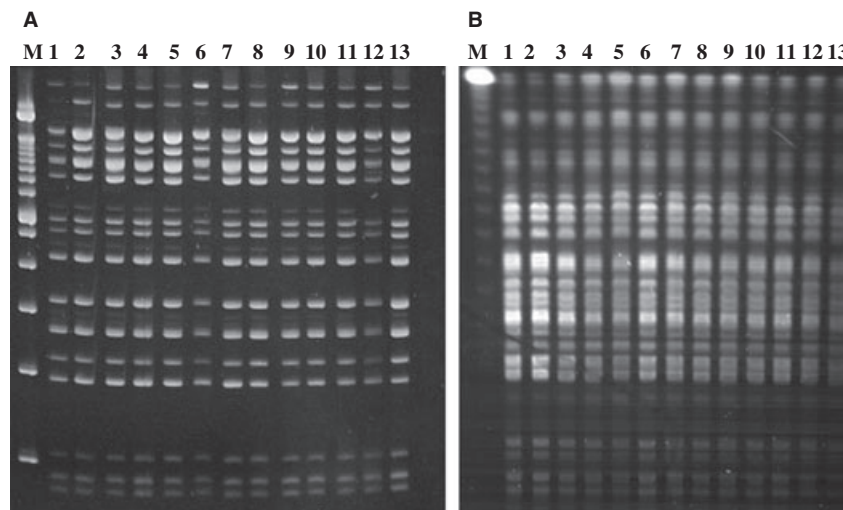


Figure 1. (A) PCR MP fingerprints for *Escherichia coli* strains. The lane designated M contained the molecular mass markers (2072, 1500, 1400, 1300, 1200, 1100, 1000, 900, 800, 700, 600, 500, 400, 300 and 200 bp). (B) REA-PFGE results for *E. coli* strains. The lane designated M contained the molecular mass markers (636.5, 582, 533.5, 485, 436.5, 388, 339.5, 291, 242.5, 194, 145.5, 97 and 48.5 kbp). The sample number is given in each lane.

increased attraction of Dr⁺ *E. coli* to maternal/fetal tissues as gestational tropism.

To our knowledge we present the first clinical/bacteriological/histopathological evidence that Dr⁺ *E. coli* may cause interstitial tubulonephritis with chronic pyelitis in humans. We also show for the first time the usefulness of a PCR MP technique, based on using low denaturation temperatures during LM PCR for bacteriological investigations at autopsy.^{1,2}

On admission the patient had no signs or symptoms of pyelonephritis. For an asymptomatic patient, bacteriuria is defined as having two consecutive voided urine specimens with isolation of the same bacterial strain in quantitative counts of $\geq 10^5$ cfu/ml. In clinical practice, however, only one voided urine specimen is typically obtained, and treatment is usually started in women with $\geq 10^5$ cfu/ml without obtaining a confirmatory repeat culture. As antibiotic resistance may be significant in urinary isolates, antimicrobial susceptibility testing should be performed and an appropriate antibiotic selected accordingly. The likely precipitating factors of urosepsis in this patient, include, but are not limited to: delayed antibiotic therapy, *E. coli* resistance to ampicillin, the lethality of invasive Dr⁺ *E. coli*, pre-existing subclinical/chronic interstitial nephritis/pyelitis, immunosuppression/steroid use in the context of an underlying pyelonephritis, and the lack of access to the history of UTI and treatment. The possible mechanisms resulting in the unexpected death may involve both microbial and host characteristics. One of the most intriguing characteristics of Dr⁺ *E. coli* is that Dr adhesin is a gestational lethal factor, which was shown to result in the death of pregnant but not non-pregnant rodents with experimental uterine infection.⁹ Gestational age-dependent infection of pregnant women by Dr⁺ *E. coli* could be explained by a temporary increase in the expression of tissue receptor decay accelerating factor (DAF), also called CD55, which is upregulated by progesterone.¹⁰ An increased density of DAF receptors in the colon and urinary tract may allow for increased colonization by Dr⁺ *E. coli*, thereby temporarily increasing the risk for UTI.³

In renal transplant patients, pyelonephritis caused by *E. coli* expressing P adhesins results in injury/renal failure, whereas Dr *E. coli* is associated with septicaemia.¹¹ The occurrence of *E. coli* strains (such as the one described here), which simultaneously bear Dr and P adhesins, and are resistant to antibiotics, could represent an unusually high-risk situation to a pregnant patient. It is well recognised that urosepsis in non-pregnant patients is associated with virulent *E. coli* bearing P adhesins. However, similar data for pregnancy is lacking.

The biological complexity and molecular epidemiology of pyelonephritis suggest that obstruction is not the only factor leading to the risk of developing gestational UTI.

Urine stasis and obstruction may favour infection in the presence of non-virulent random Gram-negative and Gram-positive species. Respectively, urosepsis in pregnant women may be associated with a less virulent random species, but this is inconsistent with the findings in the current case. Genotyping methods are not frequently used for postmortem bacteriology.^{12–14} One of these genotyping methods is the recently developed PCR MP technique, which is based on the use of low denaturation temperatures during LM PCR.^{1,2} Therefore, with improved diagnostic possibilities such as PCR and genotyping techniques, forensic pathologists could, in close association with the field of microbiology, make a significant contribution to the detection of infectious agents. The combined antibiotic resistance and lethal potential of *E. coli* bearing Dr and P adhesins requires further study. The clinical risk to the patient, timing of treatment and choice of antibiotic for the treatment of resistant microorganisms, and the role of immunosuppression and steroid use in pregnant women with pyelonephritis requires serious reevaluation.³

Disclosure of interests

The authors have no conflict of interest to disclose.

Contribution to authorship

Study concept and design by A Sledzińska, A Samet, BN, SN and JK. Genotyping and DNA analysis was completed by JK, AM and BK. Interpretation of data was done by JK, A Sledzińska, A Samet, BN and SN. Drafting of the manuscript was completed by JK and BN and critical revision of the manuscript was completed by BN, SN, A Samet and JK. Histopathology and autopsy was performed by ZJ, JK, BN and SN obtained funding. Administrative, technical and material support was undertaken by BN, SN and JK. Study supervision was performed by A Samet, JK and BN.

Details of ethics approval

Protocol NKEBN/77/2010.

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References

- 1 Krawczyk B, Samet A, Leibner J, Śledzińska A, Kur J. Evaluation of a PCR melting profile (PCR MP) technique for bacterial strain differentiation. *J Clin Microbiol* 2006;44:2327–32.
- 2 Adamus-Bialek W, Wojtasik A, Majchrzak M, Sosnowski M, Parniewski P. (CGG)₄-based PCR as a novel tool for discrimination of uropathogenic *Escherichia coli* strains: comparison with enterobacterial repetitive intergenic consensus-PCR. *Clin Microbiol* 2009;47:3937–44.
- 3 Nowicki B. Urinary tract infection in pregnant women: old dogmas and current concepts. *Curr Infect Dis Rep* 2002;4:529–35.
- 4 Nowicki B, Truong L, Moulds J, Hull R. Presence of the Dr receptor in normal human tissues and its possible role in pathogenesis of ascending urinary tract infection. *Am J Pathol* 1988;133:1–4.
- 5 Hart A, Nowicki BJ, Reisner B, Pawelczyk E, Goluszko P, Urvil P, et al. Ampicillin-resistant *Escherichia coli* in gestational pyelonephritis: increased occurrence and association with the colonization factor Dr adhesin. *J Infect Dis* 2001;183:1526–9.
- 6 Goluszko P, Niesel D, Nowicki B, Selvarangan R, Nowicki S, Hart A, et al. Dr operon-associated invasiveness of *Escherichia coli* from pregnant patients with pyelonephritis. *Infect Immun* 2001;69:4678–80.
- 7 Johnson JR, Skubitz KM, Nowicki BJ, Jacques-Palaz K, Rakita RM. Nonlethal adherence to human neutrophils mediated by Dr antigen specific adhesins of *Escherichia coli*. *Infect Immun* 1995;3:309–16.
- 8 Goluszko P, Moseley S, Truong LD, Kaul A, Nowicki S, Nowicki B. Development of experimental model of chronic pyelonephritis with *Escherichia coli* O75:K5:H- bearing Dr fimbriae: mutation in the dra region prevented tubulointerstitial nephritis. *J Clin Invest* 1997;99:1–11.
- 9 Nowicki B, Fang L, Singhal S, Nowicki S, Yallampalli C. Lethal outcome of uterine infection in pregnant but not in nonpregnant rats and increased death rate with inhibition of nitric oxide. *Am J Reprod Immunol* 1997;38:309–12.
- 10 Kaul A, Goluszko PG, Hart A, Martens M, Pham T, Nowicki S, et al. Rapid cycle changes in density and accessibility of endometrial ligands for *Escherichia coli* Dr fimbriae. *Infect Immun* 1996;64:611–5.
- 11 Rice JC, Peng T, Kuo YF, Pendyala S, Simmons L, Boughton J, et al. Renal allograft injury is associated with urinary tract infection caused by *Escherichia coli* bearing adherence factors. *Am J Transplant* 2006;6:2375–83.
- 12 Gerber JE. Acute necrotizing bacterial tonsillitis with *Clostridium perfringens*. *Am J Forensic Med Pathol* 2001;22:177–9.
- 13 Tsokos M. Fatal Waterhouse-Friderichsen syndrome due to *Ewingella americana* infection. *Am J Forensic Med Pathol* 2003;24:41–4.
- 14 Türk EE, Spermhake JP, Tsokos M. Pseudomembranous colitis with fatal outcome in a 43-year-old man. *Leg Med* 2002;4:246–50.