Nucleotide sequence analysis of NPS-1 β-lactamase and a novel integron (In1427)-carrying transposon in an MDR Pseudomonas aeruginosa keratitis strain

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Sir,

The gene encoding NPS-1 β-lactamase (blaNPS-1) was first reported on a self-transmissible plasmid of clinical isolates of Pseudomonas aeruginosa.1 The enzyme can hydrolyse carbencillin, azlocillin, cefoperazone and cefsulodin, but cannot effectively hydrolyse more stable β-lactams (cefotaxime, ceftazidime, ceftriaxone, monobactam and imipenem).1 Furthermore, blaNPS-1 was encoded on a mobile element of a large conjugative plasmid (p84) isolated from an activated sludge,2 which shows that blaNPS-1 may rapidly transfer between different species from different sources.

In the current study, we report an NPS-1-associated Tn3-like complex transposon in a microbial keratitis isolate of P. aeruginosa.

An MDR P. aeruginosa strain (PA34) was isolated from a microbial keratitis patient in India (MIC in Table S1, available as Supplementary data at JAC Online). WGS was performed using MiSeq (Illumina, San Diego, CA, USA) generating 300 bp paired-end reads and a Nextera XT DNA library preparation kit (Illumina) was used to prepare the library. CLC Genomics Workbench 10.0 (QIAGEN Bioinformatics, Aarhus, Denmark) was used to assemble the raw reads. A total of 2 365 568 trimmed reads were assembled into 75 contigs >10 kb with an average coverage of 85×. The nucleotide sequence of the transposon is available under the GenBank® accession number MF487840. Itinerary BLASTn® has revealed that the draft genome of PA34 carries a class 1 integron that is located within a Tn3-like transposon.3 The integron has a locus containing two gene cassettes comprising genes for trimethoprim (dfrA15) and chloramphenicol (cmA1) resistance. Analysis of the nucleotide sequence flanking these two cassettes revealed important characteristics of gene cassettes which included attC sequences downstream of the dfrA15 and cmA1 genes. See Figure 1a and b. The dfrA15 of PA34 is identical to that of many enteric bacteria,4 suggesting this may be their source of origin. Furthermore, the cmA1 gene was similar to a chromosomal chloramphenicol efflux transporter (U12338.3). This indicates that acquisition of a chromosomal gene may have occurred in the integron as a gene cassette. As trimethoprim and chloramphenicol are not a choice in treatment regimens for P. aeruginosa infections owing to its natural resistance, the presence of dfrA15 and cmA1 gene cassettes may be of less concern. However, their presence in isolates from the eye, which is a site of interaction of a heterogeneous microbial community, may indicate the potential source of transferable resistance traits. This integron has a new array of gene cassettes and was named In1427, a novel class 1 integron.5 A characteristic of the integron was truncated by IS6100, which has also been reported to disrupt an integron in Klebsiella oxytoca.6 This suggests that IS6100 may be responsible for introduction of In1427 into the transposon of PA34.

The transposon also possesses three other antibiotic resistance genes: two aminoglycoside-modifying enzymes (AMEs) and one β-lactamase (NPS-1) (Figure 1a). The first AME is APH(3′)-Ib, identical to strA from Pseudomonas sp. B13, and the second AME is APH(6)-Id, identical to strB from Mycobacterium abscessus subsp. ballei F1725 plasmid BRA100. Tauch et al.2 have described that the strA-strB gene pair in Tn5393c was highly transferable among bacteria isolated from plants, animals and humans. However, the original structure of Tn5393c, which was found first in the R plasmid pRAS2 from the fish pathogen Aeromonas salmonicida subsp. salmonicida (AF262622.1),7 was interrupted in PA34 by a 5735 bp region that also included another resistance gene (blaNPS-1). The segment was inserted between the tnpR and strA-strB pairs (Figure 1c). An ISPsy42 (Tn3 family) was found in the region between Tn911 and 15 439 bp of the transposon. This suggests that Tn5393c may have the capacity to be integrated by other mobile elements that may be incorporated into resistance genes (blaNPS-1 in this case). A putative mobile element (Tn6205) was found between two resolvase genes and was associated with the strA, strB and blaNPS-1 genes (Figure 1a). The Tn6205 also disrupted the second tnpA. This genetic organization of a Tn3-like transposon of PA34 suggests that insertion and recombination of antibiotic resistance genes may have occurred in multiple steps and dissemination of antibiotic resistance may have occurred between distantly related bacteria.

blaNPS-1 was inserted between tnpR and strA and its genetic environment is different in PA34 than that previously reported in the plasmid pB42 (Figure 1d). In PA34, blaNPS-1 is associated with Tn3-like transposons along with aminoglycoside resistance genes. This suggests that blaNPS-1 can be found in different genetic environments and may have different sources in the environment. The nucleotide sequence of blaNPS-1 was 100% similar to plasmid pMLH50.
(from a clinical isolate), but only 99.74% similar to plasmid pB4 (from environmental isolates) and a draft genome sequence of Pseudomonas stutzeri 40D2 (MWUI01000033.1) (M. S. R. Shuvo, S. Bashar, M. Karmaker, N. Chauhan, P. Nilawe, M. Sultan and M. A. Hossain, unpublished data). This suggests that blaNPS-1 is circulating in environmental resistance gene pools and can be acquired and maintained in clinical isolates. Further research will help ascertain the exact location of the transposon in the PA34 genome.

In conclusion, the current study shows that an ocular isolate of P. aeruginosa may act as a reservoir of resistance genes.

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Figure 1. Schematic representation of the features of the Tn3 transposon of PA34. The genes of each segment are shown by arrows, indicating assigned functions and the direction of transcription: grey with black border indicates resistance genes and white with black border indicates genes responsible for transposition. (a) Map of the major features of the complete transposon. Mobile elements are indicated by the lines below the sequence. The black solid triangles at the end of lines indicate a repeat region of the elements. A single triangle appearing on the mobile element line means it is truncated. The dotted line indicates the position of the class 1 integron. The schematic is drawn to scale by Easyfig9 with minor modification. (b) Details of the arrangement of the resistance gene cassette in the integron (Tn1427). Pint1, promoter for transcription of gene cassettes; intI1, integrase gene; attI1, integration site; dfrA15, type I dihydrofolate reductase; cmlA1, chloramphenical transporter; attC, cassette boundary (59-base element); qacEA1, partially deleted gene that encodes quaternary ammonium compound resistance; sul1, sulphonamide resistance; Δarf5, truncated gene of unknown function. Numbers correspond to number of bp (map not drawn to scale). (c) A genetic map of Tn5393c of Aeromonas salmonicida subsp. salmonicida (AF262622.1) (5670 bp) and the homologous structure in Tn3-like transposon of PA34. The segment in the rectangular box represents genes inserted in the original structure of Tn5393c and the downward arrow indicates the location of homologous genes. (d) The genetic environment of the blaNPS-1 gene region from plasmid pB4. Numbers correspond to number of bp.

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None to declare.

Supplementary data
Table S1 is available as Supplementary data at JAC Online.

References
Meningitis due to autochthonous acute infection with hepatitis E virus in a chef: a case report

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Sir,

Hepatitis E virus (HEV) is a well-known cause of acute hepatitis worldwide and a member of the genus Hepevirus in the Hepeviridae family.1 In Western European countries, hepatitis E infection is an emerging disease, where an increasing number of autochthonous hepatitis E cases have been reported.1 In these countries HEV infection is typically caused by genotype 3, mainly affects middle-aged or elderly males and is zoonotic with a porcine primary host.1–3 HEV infection usually leads to acute hepatitis but it may evolve to a chronic state, especially in immunosuppressed individuals.2 Like other viral hepatitis, several extra-hepatic manifestations have been described in association with hepatitis E, including neurological disorders such as Guillain–Barré Syndrome, neuralgic amyotrophy and encephalitis.4–6 Herein, to our knowledge, we report for the first time in Spain a case where simultaneous acute hepatitis and meningitis occurred in a patient infected by HEV, and in whom HEV RNA was detected and characterized in serum and CSF.

A middle-aged patient, working as a chef in Galicia (in the northwest of Spain), was hospitalized due to a 3-day history of fever, arthralgia, myalgia and intense headache. He did not complain about earache, cough, expectoration, abdominal pain, nausea, vomiting, change in bowel habit or lower urinary tract symptoms. He denied recent trips abroad. Upon arrival he presented with a temperature of 37.4°C. General physical examination revealed the presence of hepatomegaly. The patient was fully conscious and oriented. Nuchal rigidity and Kernig’s signs were absent. The rest of the neurological examination was also normal. At admission, blood tests detected slight neutrophilia without leucocytosis and elevation of AST (277 IU/L; reference 9–38 IU/L) and GGT (268 IU/L; reference 5–46 IU/L) and alanine aminotransferase (ALT) (441 IU/L; reference 9–38 IU/L) and GGT (344 IU/L; reference 9–38 IU/L). Coagulation tests were normal. Urinalysis showed no evidence of infection. Chest X-ray and brain computed tomography revealed no alterations. CSF examination showed a mild lymphocytic pleocytosis (34 cells/mm³; 95% lymphocytes; no red blood cells) with a slight hyperproteinorrachia (0.49 g/L; reference 0.15–0.45 g/L) and no glucose consumption.

After 5 days of hospitalization significant worsening in blood tests was detected: AST 880 IU/L, ALT 2725 IU/L, GGT 607 IU/L and total bilirubin 1.3 mg/dL (reference 0.2–1.2 mg/dL). Abdominal ultrasound showed severe hepatic steatosis and fibrosis, probably related to previous chronic and excessive alcohol consumption. Standard cultures of CSF remained negative and several infectious diseases responsible for neurological symptoms (Lyme disease, syphilis, mycoplasma, Brucella, Coxiella, Leptospira, HIV, herpesviruses, measles, mumps, parvovirus B19, enteroviruses) and acute hepatitis (hepatitis A, B, C) were excluded by nucleic acid and serology assays performed on serum and CSF. In contrast, HEV infection was diagnosed by detection of anti-HEV IgM [index >1.2; threshold value 1.0 in ELISA test (DIA.PRO, Milan, Italy)] in serum and HEV RNA in serum and CSF. HEV RNA was recovered