Whole Genome Sequencing as a Tool for Characterization of Neonatal Meningitis Escherichia coli

Michal Andrezál[1], Lukáš Pazderka[2], Tomáš Szemes[1,3], Hana Drahovská[1,3]
[1]Department of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia
[2]AnalytX s.r.o., Department of Clinical Microbiology Trnava, Slovakia
[3]Science Park, Comenius University in Bratislava, Slovakia

Neonatal meningitis is a life-threatening disease with high mortality rate and possible neurological sequelae with neonatal meningitis *E. coli* strains (NMEC) being of the primary gram-negative pathogen causing the disease. NMEC are often equipped with various virulence factors which help them to evade immune system of the host and translocate through the blood-brain barrier. In this study, we use whole genome sequencing for analysis of NMEC strains isolated from two patients together with one isolate sampled in hospital environment. All strains were characterised as sequence type 95, possessing O45:H7:K1 or O1:H7:K1 serotypes which are among the most prevalent serotypes worldwide. The patient strains were genetically very similar but differed from the environmental isolate. This proved that the environmental strain was not the source of infection. The strains contained several virulence factors specific for NMEC.
Keywords: neonatal meningitis, NMEC, genotyping, MLST, genome sequencing

Celogenómové sekvenovanie ako nástroj na charakterizáciu kmeňov *Escherichia coli* spôsobujúcich neonatálnu meningitídu
Neonatálna meningitída je závažné, život ohrozujúce ochorenie, ktoré je v mnohých prípadoch spôsobené kmeňmi *E. coli*. Kmene *E. coli* vyvolávajúce meningitídu (NMEC) sú pre patogénny proces vybavené množstvom virulenčných faktorov. V tejto práci sme stanovili celogenómovú sekvenciu kmeňov NMEC izolovaných z dvoch pacientov s meningitídou a jedného kmeňa izolovaného z povrchového steru v novorodeneckom oddelení. Všetky kmene boli zaradené do svetovo rozšíreného sekvenčného typu 95 a sérotypov O45:H7:K1 a O1:H7:K1. Kmene izolované od pacientov boli navzájom geneticky veľmi podobné, avšak líšili sa od kmeňa izolovaného z prostredia, vďaka čomu môžeme predpokladať, že tento kmeň nebol príčinou vzniku epidémie. V kmeňoch sme detegovali množstvo virulenčných faktorov špecifických pre kmene NMEC.
Kľúčové slová: neonatálna meningitída, NMEC, genotypizácia, MLST, celogenómové sekvenovanie

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Introduction

Meningitis is one of the major causes of mortality and morbidity in neonates. Together with group B streptococci, neonatal meningitis-causing *Escherichia coli* (NMEC) is the leading cause of this disease worldwide[(1)]. Mortality rates can reach up to 40%, while survivors often suffer with severe neurological sequelae and may develop serious neurological disorders[(2)]. Pathogenesis of NMEC meningitis is complex, encompassing the need for bacterial translocation to the bloodstream, and crossing of the blood-brain barrier[(1)]. To accomplish this, the NMEC are equipped with a wide variety of virulence traits such as adhesins, iron acquisition systems, invasins, toxins, and serum resistance genes. These factors can be encoded by bacterial chromosome or are carried via plasmids[(1,3)]. One of the most common virulence factors in NMEC strains is K1 antigen. It is a homopolymer of N-acetylneuraminic (sialic) acid and functions as a molecular mimicry[(4)].

The aim of this study was molecular typing of four NMEC isolates. Three strains were isolated from two diseased neonates and one isolate originated from the surface in the neonatal unit of the same hospital.

Materials and Methods

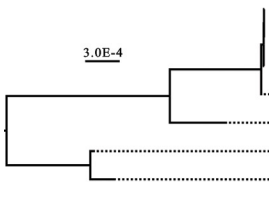
Isolation and cultivation of bacterial strains

Strains used in this study were isolated in the Neonatal Department of Trnava University Hospital (Slovakia). KMB-1033 and KMB-1034 were isolated from Patient 1, KMB-1032 was isolated from Patient 2., and KMB-1031 strain was isolated from the surface swab of the hospital environment. Strains were grown on Columbia agar 5% Sheep Blood or in Luria-Bertani (LB) medium.

DNA isolation and NGS library preparation

Nucleic acid isolation was performed by Higher Purity™ Bacterial Genomic DNA Isolation Kit (CanvaxBiotech). DNA

Figure 1. Phylogenetic tree of four isolates analysed in this study (KMB-1031 – KMB-1034, blue) and three reference NMEC strains (red). The tree was generated by PATRIC, an online tool with program set-up to compare 1000 genes across selected genomes. Bootstrap values were not lower than 98. The origin of the strains: S88 France – acc. no. NC_011742; IHE3034 Finland – acc. no. NC_017628; Rs218 USA – acc. no. NZ_JWZW00000000, CSF – cerebrospinal fluid



	Country	Source	Serotype	MLST	CH type	cgMLST	beta-lactamases class A	beta-lactamases class C
KMB-1034	Slovakia	hemoculture	O45:H7:K1	95	38/54	177333	TEM-1	EC-5
KMB-1033	Slovakia	CSF	O45:H7:K1	95	38/54	177330	TEM-1	EC-5
KMB-1032	Slovakia	CSF	O45:H7:K1	95	38/54	182004	TEM-1	EC-5
S88	France	CSF	O45:H7:K1	95	38/54	N/A	-	EC-5
KMB-1031	Slovakia	surface swab	O1:H7:K1	95	38/30	147572	TEM-1	EC-5
IHE3034	Finland	unkown	O18:H7:K1	95	38/18	89611	-	EC-5
Rs218	USA	CSF	O18:H7:K1	95	38/18	130631	-	EC-5

concentration was measured by Qubit (Invitrogen). Genomic libraries for sequencing were prepared by Nextera XT DNA Prep Kit (Illumina), purified on AMPure XP magnetic beads (Beckman Coulter Life Sciences) and reviewed by chip electrophoresis. Sequencing was performed on NextSeq 500™ platform using 2x150bp read lengths (Illumina) at the Comenius University Science Park.

Bioinformatics and genome analysis

Raw data was assembled into contigs by SPAdes. Assembled genomes were annotated and analysed using the PATRIC (https://www.patricbrc.org/), RAST (https://rast.nmpdr.org/) and CGE (https://www.genomicepidemiology.org/) online platforms.

Results and discussion

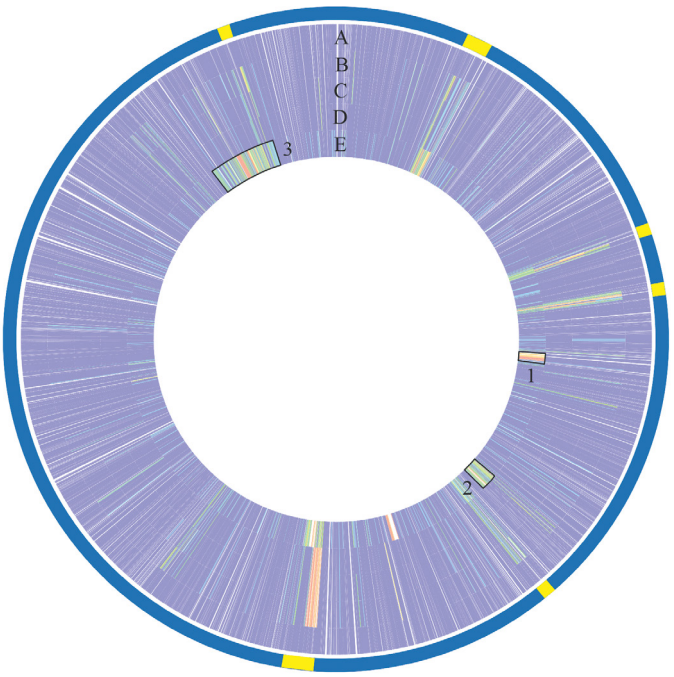
All four tested strains were identified as *Escherichia coli* based on biochemical EnteroTest 24 + latex agglutination of liquor, which is positive in *E. coli* K1 samples, and verified by PCR and MALDI-TOF methods.

Using whole genome sequencing, we obtained the average of 86 contigs with 76 times coverage per genome. All four strains were positive for K1 capsule and belonged to the MLST sequence type ST-95 which is very common among NMEC strains[5,6]. They were distinguishable only by cgMLST. Furthermore, the KMB-1031 strain isolated from environmental swab also showed difference in serotype and *fimH* fimbrial adhesin (**Figure 1**). To further distinguish the highly similar genomes, whole genome SNP analysis was applied, which confirmed strain separation into two clusters. Strains isolated from patients differed from each other in 111 to 128 SNPs. However, the environmental KMB-1031 strain was less relative to the other strains and differed in 2830 to 2865 SNPs. Our isolates were further compared with three reference NMEC genomes and we found out that the clinical strains were highly relative to *E. coli* S88 (**Figure 1**). This strain is a typical representative of the O45:H7:K1 serotype, frequently found in NMEC in Spain and reported to account for one-third of all *E. coli* neonatal meningitis cases in France in 2009[6,7]. The O1:H7:K1 serotype, seen in the strain isolated from the surface swab (KMB-1031), was the most prevalent NMEC clonal group in France between 2001 and 2013 and in the USA[5] as well.

We compared proteomes of all four strains with S88 as the reference and observed high similarity between S88 and KMB-1032, KMB-1033 and KMB-1034 strains, while lower similarity to KMB-1031, which was anticipated based on previous analyses (**Figure 2**). Main differences were seen in regions encoding for prophages, amino-acid biosynthesis, colanic acid biosynthesis, formate and nitrate metabolism, type 1 fimbriae as well as hypothetical proteins. These differences suggest that the strain isolated from the surface swab was not the cause of the meningitis outbreak in the neonatal department.

Based on the CARD database, TEM-1 and EC-5 genes which confer resistance to penicillins and cephalosporins

Figure 2. Proteome comparison of the reference E. coli S88 (A) and the KMB-1032 (B), KMB-1033 (C), KMB-1034 (D), and KMB-1031 (E) strains. Yellow rectangles highlight the areas of difference in encoding for phage proteins. Black rectangles show differences between KMB-1031 and the rest of our strains. These areas encode proteins involved in formate and nitrate metabolism (1), biosynthesis of colanic acid and some amino-acids (2), type 1 fimbriae (3), as well as many other genes, hypothetical genes and genes with unknown function. Dissimilarities in genes involved in synthesis of type 1 fimbriae was anticipated because of different fimH allele detected in this strain.



were detected in all four strains[8]. This was consistent with the ampicillin resistance (>32 mg/l) detected in KMB-1031, KMB-1033 and KMB-1034 strains. Furthermore, *E. coli* KMB-1031 showed resistance against ampicillin + sulbactam (>32 mg/l) which may be caused by the fact that sulbactam is a relatively weak inhibitor of TEM-1, or possibly a result of TEM-1 hyperproduction[9].

Presence of plasmids was detected by selection of contigs with higher coverage. In all four strains, we detected a large (~135 kb) plasmid similar to previously published pRK100 and pCERC5 plasmids belonging to the ColV group with RepFIB and RepFII origins[10]. We observed similarity in the whole plasmid sequence with the only difference that unlike pRK100 and pCERC5, plasmids from our strains did not harbour the *tetA* gene (data not shown). We observed presence of twelve virulence genes (*cia, cvaC, iroN, iss, mchF, traT, etsC, hlyF, ompT, iucC, sitA* and *iutA*) on the pRK100-like plasmid found in our strains. No differences in the presence of virulence factors localised on the pRK100-like plasmids were detected between the patient and environmental strains. The IncFIB plasmids are very prominent among ExPEC strains and other studies[11,12] have also described an association between these plasmids and genes encoding for siderophores and serum survival determinants in NMEC.

Conclusion

This paper describes an analysis of three clinical NMEC strains and one environmental swab isolate. All four strains were characterised as ST-95 possessing O45:H7:K1 and O1:H7:K1 serotypes which both belong to widely spread NMEC types in many countries. Virulence factors found in these strains could help identify specific set of genes for distinguishing NMEC from other ExPEC. Furthermore, this paper contributed to the monitoring of transmission of epidemics in a neonatal medical facility.

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doc. RNDr. Hana Drahovská, PhD.
Department of Molecular Biology
Faculty of Natural Sciences, Comenius University in Bratislava
Ilkovičova 6, 841 04 Bratislava, Slovakia
e-mail: hana.drahovska@uniba.sk