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Poster Session V

Legionella: news in clinical and molecular epidemiology

WHOLE GENOME SEQUENCING INVESTIGATION OF HOUSEHOLD WATER FILTRATORS AND COLD WATER HUMIDIFIERS AS EMERGING VEHICLES FOR PAEDIATRIC LEGIONNAIRES' DISEASE

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Objectives: Whilst paediatric Legionnaires' Disease (LD) is rare, cold water humidifiers have recently emerged as vehicle of LD in children. Household water filtrators are increasingly being used and considered safe by lay public owing to charcoal filtering and UV light activity. We report the epidemiological and molecular investigation of three humidifier associated cases using WGS.

Methods: Laboratory confirmed LD cases were investigated following reporting to health authorities. Environmental samples were recovered from domestic water systems and appliances and tested for *Legionellapneumophila* (Lp) using standard protocols. Lp strains were confirmed by standard procedures and *mip* qPCR. Lp molecular typing was performed using the ESGLI sequence-based typing (SBT) and query of the PHE SBT database. DNA extracts were subject to Whole Genome Sequencing (WGS) using Illumina technology (MiSeq) and sequence data were *de novo* assembled using CLCbio and then analysed by the SeqSphere+ software (Ridom GmbH, Münster, Germany). Strain relatedness was assessed by comparing the number of differing alleles in the Lp core genome (core genome MLST or MLST+ approach).

Results: All three cases involved healthy infants <6 months. Household humidifiers were filled with tap water directly from faucet or filtrated cold water and in all cases parents were unaware of the recommendation to use pre-boiled water. Case 1 (2012) was fatal and involved Lp ST1 which was recovered from sputum culture and the cold water humidifier (Moran-Gilad et al., Eurosurv 2012). Case 2 (2013) presented as mild LD and diagnosed by urinary antigen testing. Environmental household cultures yielded Lp ST1 from the kitchen cold water system, water dispensed from the filtrating machine, residual water drained from the machine's charcoal filter and residual water from the humidifier. Case 3 (2013) presented as severe LD and was diagnosed using sputum PCR. Lp ST1 was recovered from the kitchen cold and hot water systems, residual water from charcoal filters and residual water from the humidifier. WGS of strains recovered in Case 1 revealed identical strains. Strains from case 2 were identical and formed a distinct cluster thus confirming the hypothesis that filtrated water used to fill the humidifier harboured Lp. Case 3 strains were more diverse; strains recovered from cold water differed from humidifier strain by 29 alleles whilst strains from hot water differed by 104 alleles, thus suggesting strains from cold water were more likely to caused humidifier associated LD.

Conclusion: Filtrated household water may harbour Lp and should be pre-boiled if used to fill humidifiers in order to prevent LD. MLST+ analysis generated through Lp WGS is a promising tool for investigation of LD and increases the resolution of strain relatedness analysis amongst identical STs as well as assist in tracing Lp infection origin.