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Paper Poster Session
The complexity of antibacterial resistance mechanisms

Genomic sequencing of two Streptococcus agalactiae with high level gentamicin resistance, collected in the BSAC bacteraemia surveillance

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Background: Like all members of its genus, *S. agalactiae* ('Group B streptococcus') typically has low-level aminoglycoside resistance, reflecting poor uptake of these drugs. High-level resistance, mediated by modifying enzymes, is extremely rare, with one previous (2002) UK report, along with others from France and Argentina. From 2001-14, the British Society for Antimicrobial Chemotherapy (BSAC) bacteraemia surveillance (http://www.bsacsurv.org) has examined 1125 *S. agalactiae* from >70 sites in the UK and Ireland, with no highly gentamicin-resistant isolates found until 2014, when two were encountered at different hospitals. These were characterised.

Material/methods: Identifications were by latex agglutination, confirmed by sequencing; MICs were determined by BSAC agar dilution; sequencing was by Illumina methodology, with bioinformatic analysis using a locally-curated database of resistance determinants.

Results: Gentamicin MICs for the two isolates exceeded 1024 mg/L compared with 4-16 mg/L for other *S. agalactiae* collected in the 2014 bacteraemia surveillance. Sequencing revealed that both isolates belonged to the international ST19 clone, but were distinct, differing by 934 SNPs. Both carried *aac6'-aph2''*, explaining their gentamicin resistance, and had *gyrA*[81:S-L];*parC*[79:S-Y], accounting for high-level ciprofloxacin resistance (MICs 64 mg/L) – another trait that, in the 2014 bacteraemia collection, was unique to these two isolates. In addition, both had *tet(M)*, and were resistant to tetracycline (MIC 64 mg/L), but so too were 83.6% of all *S. agalactiae* collected under the aegis of the BSAC bacteraemia surveillance from 2001-14. One of the two isolates had *mef(E), erm(B), msr(D), lsa(E) and lnu(B)* and was highly resistant to both erythromycin and clindamycin (MICs both >128 mg/L); the second had *erm(TR)* and *lnu(C)* with erythromycin and clindamycin MICs of 2 and 0.12 mg/L, respectively. Although complete assembly has proved difficult, it is clear that *aac6'-aph2''* was transposon-borne in both isolates and that the host transposons differed from each other and from those previously published in *S. agalactiae*. Both isolates remained fully susceptible to penicillin (MICs 0.06 mg/L).

Conclusions: Multiresistant *S. agalactiae* with high-level gentamicin resistance mediated by the bifunctional AAC(6')-APH(2") enzyme have emerged or re-emerged in the UK. Despite some similarities, including exceptional fluoroquinolone resistance and carriage of *lnu* genes, encoding lincosamide nucleotidyltransferases, the two isolates and their resistance transposons were distinct. Given continued penicillin susceptibility, the clinical significance is limited but any synergy of the penicillin-gentamicin regimens frequently used in neonatal sepsis is likely to be abrogated.