Role of efflux in macrolide resistance in β-haemolytic streptococci of groups A, B, C and G collected in an Irish teaching hospital

There has been an increase in macrolide resistance in β-haemolytic streptococci (BHS) reported worldwide. Macrolide resistance rates among group A streptococci (GAS) are variable, and range from 2 to 32% in areas of the USA and Canada, from 5 to 29% within Europe, and are as high as 63% in Taiwan (Lecleq, 2002). Furthermore, a recent study in Spain found erythromycin resistance to be 26.1% in GAS, 15.7% in group B streptococci (GBS), 5.3% in group C streptococci (GCS) and 33.3% in group G streptococci (GGS) (Merino Díaz et al., 2008). Mechanisms that confer resistance to macrolide, lincosamide and streptogramin B (MLSb) antibiotics include target site modification by methylation of the 23S rRNA binding site. This mechanism is mediated by the \textit{erm} gene, which confers cross-resistance to all MLSb antibiotics. Resistance can be either inducible (iMLSb), where the methylase is produced in the presence of an inducer (such as erythromycin), or constitutive (cMLSb), where the methylase is produced constitutively. Drug efflux, which has been increasingly recognized, is mediated by the \textit{mefA/E} gene, which encodes a 12-membered transmembrane protein that pumps out 14- and 15-membered ring macrolides only, with the isolate remaining susceptible to 16-membered MLSb antibiotics (M phenotype). The third resistance mechanism occurs as a result of antibiotic inactivation. Resistance is usually conferred to only one of the three classes (M, L or S). In \textit{Enterococcus faecium} the \textit{linB} gene encodes lincosamide nucleotidyltransferase, which inactivates lincosamides, conferring resistance to clindamycin only (L phenotype). The latter phenotype has been reported in clinical isolates of GBS (Gygax et al., 2006). The only Irish data published on the prevalence of erythromycin resistance and mechanisms of resistance in BHS reviewed GAS only (Kiely et al., 2010). Surveillance data in an Irish tertiary-care centre showed an increase in macrolide resistance among BHS groups A, B, C and G recently. Erythromycin resistance increased from 10% in 2004 to 14% in 2009, while clindamycin resistance rates remained steady over the same period, 6% in 2004 and 5% in 2009. The aims of this study were to determine the prevalence of erythromycin resistance in BHS groups A, B, C and G from clinical isolates in the largest hospital in Ireland (St James’s Hospital). Furthermore, resistance mechanisms to macrolides were determined by molecular methods.

Over a 6 month period (May–October 2009), 360 BHS isolates were collected. A total of 8 isolates (2%) originated from blood cultures, 52 isolates (14%) from throat swabs and 300 (84%) from wound swabs. Phenotypic characterization was performed by double-disc diffusion testing as described by Swenson et al. (2007). Erythromycin (15 μg) and clindamycin (2 μg) discs were placed 20 mm apart. Isolates resistant to erythromycin with blunting of the clindamycin inhibition zone were of the iMLSb phenotype, isolates that demonstrated resistance to both erythromycin and clindamycin were of the cMLSb phenotype, isolates showing resistance to erythromycin without blunting of the clindamycin inhibition zone were of the M phenotype, and isolates resistant to clindamycin yet susceptible to erythromycin belonged to the L phenotype. Erythromycin-resistant isolates were screened for the presence of macrolide resistance genes \textit{erm}A subclass \textit{erm}TR, \textit{erm}B, \textit{mef}A/E by end-point PCR using a TaqCore kit (Qiagen) and sequences published by Weber et al. (2001), and clindamycin-resistant isolates were screened for the \textit{linB} gene using sequences published by Gygax et al. (2006). Cycling conditions were as follows: 1 cycle of denaturation at 94°C for 3 min; followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min and extension at 72°C for 1 min. Following amplification, products were run through 1.5% agarose gels. The expected sizes of the PCR products were 348, 616, 206 and 944 bp for \textit{erm}A subclass \textit{erm}TR, \textit{erm}B, \textit{mef}A/E and \textit{linB}, respectively.

Of the 360 BHS isolates collected, 59 were resistant to erythromycin (16.4%). The phenotypes and genotypes are summarized in Table 1. A total of 43 isolates were resistant to erythromycin and susceptible to clindamycin, 15 were resistant to both erythromycin and clindamycin, and 1 isolate was resistant to clindamycin but susceptible to erythromycin. Most isolates, 37 (62.7%), were of the iMLSb phenotype, 12 (20.3%) had phenotype cMLSb, 9 (15.3%) had the M phenotype and 1 (1.7%) the L phenotype. The iMLSb phenotype was most prevalent among GGS, the cMLSb phenotype in GBS, the M phenotype in GAS and there was one GAS isolate that displayed the L phenotype. For isolates displaying the iMLSb and M phenotypes, erythromycin resistance was low level with MICs ranging from 1 to 4 mg l⁻¹ and clindamycin MICs were of ≤1 mg l⁻¹. For cMLSb phenotypes, erythromycin and clindamycin MICs were ≥256 mg l⁻¹. Genotypic analysis showed that 37 (62.7%) isolates harboured the \textit{erm}A subclass \textit{erm}TR gene, 18 (30.5%) harboured the \textit{erm}B gene and these were most prevalent among GGS. Seven (11.9%) harboured the \textit{mef}A/E gene and this was most prevalent in GBS. No isolates possessed the \textit{linB} gene. Nine isolates of the iMLSb phenotype harboured both \textit{erm}A subclass \textit{erm}TR and \textit{erm}B genes. Also, 1 M phenotype harboured both \textit{mef}A/E genes and \textit{erm}A subclass \textit{erm}TR gene, while another M phenotype harboured both \textit{mef}A/E and \textit{erm}B genes. In eight isolates no resistance mechanisms were identified on molecular analysis, two of which were phenotype iMLSb, three cMLSb phenotype, two M phenotype and one L phenotype.
Erythromycin resistance in BHS in this Irish hospital has increased to 14% in 2009 compared to 10% in 2004. There is a predominance of iMLSB and cMLSB phenotypes mediated by the \( \text{ermA} \) subclass \( \text{ermTR} \) and \( \text{ermB} \) genes, respectively. However, the relatively high proportion of M phenotypes (11.9%) has important implications. Firstly, on the laboratory reporting of clindamycin susceptibility results, as clindamycin can be reported as susceptible, and secondly, it may influence antibiotic choice as clindamycin may still be a therapeutic option even if the organism is resistant to erythromycin.

There were eight isolates in the collection for which no resistance mechanism was found using primers as described above. These isolates may harbour mutations in genes coding for 23S rRNA or ribosomal proteins L4 and L22 and will be investigated further. Interestingly, there were nine isolates of the iMLSB phenotype that harboured both \( \text{ermA} \) subclass \( \text{ermTR} \) and \( \text{ermB} \) genes. The co-existence of both genes has been documented in the past (Bingen et al., 2000), but in contrast to findings in the present study, the isolate displayed the cMLSB phenotype. Also, 1 M phenotype harboured both \( \text{mefA/E} \) genes as well as \( \text{ermB} \) genes, and 1 M phenotype harboured \( \text{mefA/E} \) genes and \( \text{ermA} \) subclass \( \text{ermTR} \) genes. This finding implies differential gene expression as only the \( \text{mefA/E} \) gene was expressed. In conclusion, as erythromycin resistance rates in BHS have increased over the past number of years, continued surveillance is advisable, and local statistics will be of crucial value in guiding our empirical antibiotic therapy. It also highlights the role of efflux in macrolide resistance in BHS, which may have an impact on our antibiotic choice in clinical practice. The current study provides a baseline from which future trends in MLS\(_B\) resistance can be monitored.

Aliya Saara Khan, Anne Walsh and Brendan Crowley

Department of Microbiology, Central Pathology Laboratory, St James’s Hospital, James’s Street, Dublin 8, Ireland

Correspondence: Brendan Crowley (bcrowley@stjames.ie)


