The molecular epidemiology of MRSA in Malta and the description of a Maltese epidemic MRSA strain

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Malta has one of the highest incidences of meticillin-resistant Staphylococcus aureus (MRSA) in Europe. However, there is only limited typing data available. In order to analyse the local situation, forty-five isolates from the Mater Dei Hospital in Malta, were characterised using diagnostic microarrays.

The most common strain was ST22-MRSA-Iv, also known as UK-EMRSA-15 (30 isolates). Sporadic strains included ST3639-MRSA-II (UK-EMRSA-16, two isolates), PVL-positive ST80-MRSA-IV (European Clone, one isolate), ST228-MRSA-I (Italian/South German Epidemic Strain, one isolate) and ST239-MRSA-III (Vienna/Hungarian/Brazilian Epidemic Strain, one isolate).

Ten isolates belonged to a novel clonal complex 5, spa-type 0022 strain. This strain carries genes from a SCCmec IV element (mecA, delta mecR, ugdH, tsa, ccrA2 and ccrB2), novel alleles of ccrA/II and a fusidic acid resistance element Q6GD50 (BS718571.1:SA0043). It also harboured the gene for enterotoxin A (sea) and the ege enterotoxin locus, as well as (in nine out of ten isolates) genes encoding the toxic shock syndrome toxin (tssl) and enterotoxin C and L (see, sel). While the presence of the other MRSA strains suggests foreign importation because of intense travel activities between Malta and various European countries, so far this strain appears to be restricted to Malta yet.

Introduction: Similar to other Southern European countries, Malta has a very high prevalence of MRSA. However, to our knowledge there is only one published study describing typing data of MRSA isolates from Malta (Gould et al., 2008) indicating the presence of ST22-MRSA-IV, of ST3639-MRSA-II and of another strain which had a novel PFGE pattern, but which was not described in further detail.

In order to gain a better understanding of the Maltese MRSA population, the present study aimed to perform a comprehensive molecular characterisation of Maltese MRSA isolates recovered from patients in the Mater Dei Hospital in Msida, Malta, over a four-month period, using diagnostic DNA microarrays.

Methods: Between October 2008 and January 2009, 45 MRSA isolates were randomly collected from the Bacteriology Laboratory of Mater Dei Hospital in Msida, Malta, and characterised using routine methods for identification and susceptibility testing. Principles and details of assay procedures, have been published previously (Moncke et al., 2008). In short, staphylococcal cultures were grown overnight on Columbia blood agar and treated with lysostaphin, lysozyme and riboflavine A. Following this, samples digested with proteinase K and chromosomal DNA was purified using QIAGEN’s QIA DNA kit. Microinterrogus-mediated DNA microarrays (ASP system by CLONDIAG) were used which covered 334 target sequences. This included species markers, resistance and virulence genes, genes encoding microbial surface components recognizing adhesive matrix molecules of the host (MSCRAMMs) as well as SCCmec, capsule and arg- type typing markers. All targets were amplified in a linear primer extension reaction using a single primer per target only in order to facilitate a multiplex reaction covering all targets simultaneously. Within this step, bovin-16S-rTP was incorporated into the amplimers which then hybridised to the array. This was followed by washing and subsequent addition of Horseradish-peroxidase-streptavidin conjugate SeraBan Green precipitating dye (Seracruz, Heidelberg, Germany). An image of the array was recorded and analysed using a designated reader and software (ImageSoftware, licensed, both by CLONDIAG).

MLST and spa typing were performed according to standard protocols. SCCmec typing was performed on these two isolates using four multiplex PCRs to identify (i) the mec complex type (class A, B, and C) and K. Kondo et al., 2007), (ii) the ccr complex type (ccrA1B, ccrA2B, ccr4A1), and ccrA2 (Kondo et al., 2007), (iii) the tsa region (Olivera et al., 2002) and (iv) the SCCmec IV subtype (SCCmec IVa, IVb, IVc, IVd, IVe, IVf) (Methénos et al., 2007). Additional primers were designed to amplify and sequence a novel vir allele in CSSCCs (see below) based on sequences of ccrA5, ccrF, ccrD and the putative fusidic acid resistance gene Q6GD50. Their sequences can be provided on request.

Strain affiliations of Maltese MRSA isolates: The most common strain (Figure 1) was ST22-MRSA-IV, “Baruniuk/UK-EMRSA-15” (30 isolates, 66.7%). Sporadic strains included ST3639-MRSA-II (UK-EMRSA-16, two isolates, 4.4%), PVL-positive ST80-MRSA-IV (European Clone, one isolate, 2.2%), ST228-MRSA-I (Italian/South German Epidemic Strain, one isolate, 2.2%) and ST239-MRSA-III (Vienna/Hungarian/Brazilian Epidemic Strain, one isolate, 2.2%). All these strains are widespread in Europe. Their occurrence in Malta might be related to the high number of tourists from the U.K. as well as from continental Europe on that island.

Ten isolates (22.2%) belonged to a novel clonal complex 5, spa-type 0022 strain. This strain has not previously been described, but the novel PFGE pattern mentioned in an earlier study on MRSA in Malta might correspond to that strain.

Characterisation of a Maltese MRSA strain: Two out of ten isolates were spa typed and identified as 0022. One of them was subjected to MEST and yielded ST149.

SCCmec typing confirmed the presence of SCCmec IVa. In addition, isolates yielded hybridisation signals with ccrA3 (but not with ccrB3), PCR yielded amplifiers for ccrB3, but not for ccrA3. Further analysis revealed that the sequence of this amplifier had 99% identity to the cassette chromosome recombinase genes ccrA3/IVh/IVe/IVg/PBP2 shown to be present in an ACME-positive Staphylococcus aureus isolate from China (GenBank accession number EU934095.1, Pi et al., 2009). However, all Maltese CSSCs were ACME-negative.

All ten CSSCs MRSA isolates yielded hybridisation signals with probes for the putative fusidic acid resistance element Q6GD50, which was also in accordance to phenotypic fusidic acid resistance. All isolates harboured the beta-lactamase-encoding (blaZ, blaZ, blaZ, blaZ). Two isolates were positive for cemC and, in one isolate toeK was detected. All CSSCs were PVL-negative. They all carried sea and the ege locus. Nine out of ten CSSCs MRSA isolates harboured tssl, sac and sel. Genes sak and scw were present, whereas chp was absent. Genes encoding exfoliative toxins (sea, eta, etb, edh) or epithelial cell differentiation inhibitors (edfb, edfe, edfc) were also absent.

In conclusion this study has provided an insight into epidemiological and molecular characteristics of MRSA in Malta. In addition to the presence of pandemic strains such as ST239-MRSA-III or ST22-MRSA-IV, there is also a locally important MRSA clone apparently endemic to Malta. Factors which determine whether a clone remains genetically restricted or achieves pandemic spread still need to be determined.

Figure 1: Scan of a processed array after hybridisation with a typical Maltese CC5-MRSA. Typical genetic markers (see text) are highlighted.

Literature:

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