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Infection prevention-2018-Application of high resolution melting to methicillin resistant Staphylococcus aureus and Shigella sonnei genotyping for epidemiological purposes- Waleed A Mazi- Taif- Kingdom of Saudi Arabia -

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Introduction: High resolution melting (HRM) analysis has been used in laboratory medicine as acurate, rapid and cost effective scheme method. Methicillin resistant Staphylococcus aureus (MRSA) infections impose huge risk to public health in healthcare and community settings worldwide. Shigella sonnei has been predominantly responsible for dysentary worldwide. The organism has only one serotype and is genetically homogeneous. We evaluated MRSA spa typing and introduced new tools for Shigella sonneil genotyping using HRM analysis for epidemiological purposes.

Methods: Fifty clinical MRSA isolates were selected randomly from Scotland, Brazil, Sudan and Saudi Arabia. Methicillin- resistant phenotype was determined in accordance with BSAC standards using the Vitek 2system. Ten Shigella sonnei DNA samples were provided by Institut Pasteur, France. Primers for the polymorphic X region of the spa gene and the six single nucleotide polymorphisms (SNPs) within kduD, deoA, emrA, fdX and menF were amplified by colony PCR using the SensiMix HRM kit, and the melting temperature (Tm) and melting curves of the amplicons were analyzed in close tubes using a Rotor- Gene 6000 instrument.

Staphylococcus aureus is a major cause of hospital-acquired infections worldwide. Increased frequency of methicillin-resistant Staphylococcus aureus (MRSA) in hospitalized patients and possibility of vancomycin resistance requires rapid and reliable characterization of isolates and control of MRSA spread in hospitals. Typing of isolates helps to understand the route of a hospital pathogen spread. The aim of this study was to investigate and compare genotypic and phenotypic characteristics of MRSA samples on three different geography locations. In addition, our aim was to evaluate three different methods of MRSA typing: spa-typing, agr-typing and GenoType MRSA. We included 104 samples of MRSA, isolated in 3 different geographical locations in clinical hospitals in Zagreb, Mostar, and Heidelberg, during the period of six months. Genotyping and phenotyping were done by spa-typing, agr-typing and dipstick assay GenoType MRSA. We failed to type all our samples by spa-typing. The most common spa-type in clinical hospital Zagreb was t041, in Mostar t001, and in Heidelberg t003. We analyzed 102/104 of our samples by agr-typing method. We did not find any agr-type IV in our locations. We analyzed all our samples by the dipstick assay GenoType MRSA. All isolates in our study were MRSA strains. In Zagreb there were no positive strains to PVL gene. In Mostar we have found 5/25 positive strains to PVL gene, in Heidelberg there was 1/49. PVL positive isolates were associated with spa-type t008 and agr-type I, thus, genetically, they were community-associated MRSA (CA-MRSA). Dipstick assay GenoType MRSA has demonstrated sufficient specificity, sensibility, simple performance and low cost, so we could introduce it to work in smaller laboratories. Using this method may expedite MRSA screening, thus preventing its spread in hospitals.

This work is partly presented in 13th World Congress on INFECTION PREVENTION AND CONTROL, December 14-15, 2017, Rome, Italy

Staphylococci are among the most important causes of both hospital- and community-acquired infections worldwide. It is well known that methicillin-resistant Staphylococcus aureus (MRSA), like methicillin-sensitive Staphylococcus aureus (MSSA) could colonize or infect patients. MRSA strains were not found to be more virulent than MSSA strains and to cause the same spectrum of infections. S. aureus causes superficial and deep skin and soft tissue infections, bacteremia, endocarditis, osteomyelitis, pneumonia, food poisoning, toxic-shock syndrome and staphylococcal scaled skin syndrome. In the early 1950s, acquisition and spread of β-lactamase-producing plasmids decreased the effectiveness of penicillin for treating S.aureus infections. Methicillin, modified penicillin, designed to resist the destructive action of the staphylococcal β-lactamase, became available for therapeutic use in 1959. However, MRSA was identified in 1960s. The resistance was a result of S. aureus's acquiring the mecA gen, which encodes for an altered penicillin-binding protein gen (PBP2a). It was not blocked by methicillin and could replace the other PBPs, thus allowing the survival of S.aureus in the presence of methicillin. As opposed to the penicillinase gene, mecA does not reside on a plasmid but on the chromosome, embedded in a large mobile genetic element called Staphylococcal Chromosome Cassette mec or SCCmec. The presence of PBP2a means MRSA is not only resistant to methicillin but also to all β-lactam antibiotics, including synthetic penicillins, cephalosporins and carbapenems. By the early 1960s, European hospitals were reporting outbreaks of MRSA infections. In Bosnia-Herzegovina, we noted an increment of MRSA infections in the early 1990s, with the beginning of the war. Data from the European Antibiotic Resistance Surveillance System (EARSS) showed a rising trend of MRSA infections until 2005, with the proportion of MRSA infections varying from less than 1% in the northern to 50% in southern European countries. This striking difference is probably due to differences in antibiotic use or in the implementation of measures to control MRSA spread in hospitals. MRSA has been linked for many years- with hospital stay, homes for the elderly and infirm, and similar institutions. However, in 1990s, community-associated MRSA (CA-MRSA) has appeared, with a large number of characteristics different from previously known hospital acquired MRSA (HA-MRSA). HA-MRSA is mainly multi-resistant, and the choice of antibiotics for treating such infections is limited to glycopeptides and linezolid. Furthermore, HA-MRSA mainly causes serious infections in patients with weak immune system, after long-term hospitalization, long-term use of antibiotics, etc.. In previous years, strains have emerged with an intermediate susceptibility or full resistance to vancomycin (VISA and VRSA, respectively), the antibiotic that for two decades represented the drug of choice for treating MRSA infections. The multidrug-resistant phenotype of MRSA strains and their intrinsic β-lactam resistance make them difficult and costly to cure.

Results: Fifteen spa types detected each had a distinct melting temperature (Tm) that unambiguously assigned 44 isolates. Both t008 and t2770, as well as t311 and t021 spa types, shared the same Tm. The first set run separated lineages I, II and III with distinctive melting curves and the Tm of each allele was at least a half degree away from that of other alleles. The second set run distinguished the sublineages IIIa, IIIb and IIIc with distinctive melting curves.

Conclusion: HRM analysis is acurate, rapid and cost effective scheme method for identification of MRSA and Shigella sonnie for epidemiological purposes

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Biography

Waleed A Mazi is a regional Director for Infection Prevention and Control, King Abdul Aziz Specialist Hospital, Taif – Saudi Arabia. He also worked in Philosophy of Medical Science, Clinical Microbiology, Karolinska Institutet, Sweden. He has published international articles on prevention of central line –associated bloodstream infection, WHO- Hand Hygiene implementation program, prevention sharp injuries in healthcare settings and molecular genotyping for epidemiological purposes and participated as a poster and oral presenters in many international conferences.

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