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Diversity of accessory genome of human and livestock-associated ST398 methicillin resistant *Staphylococcus aureus* strains

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ABSTRACT

Background: Molecular typing of methicillin-resistant *Staphylococcus aureus* (MRSA) has documented the diversity of the genetic background of strains associated with healthcare (HA-MRSA), community (CA-MRSA) and livestock (LA-MRSA). The accessory and core-variable genomes of those strains however remain largely unknown.
Objective: To compare the genetic background and accessory and core variable gene content of ST398 LA-MRSA strains with those of HA-and CA-MRSA strains from the same region.
Methods: Representative strains of HA- (n = 21), CA- (n = 13) and ST398 LA-MRSA (n = 18) were selected from Belgian National Reference Laboratory collections. The accessory and core-variable genomes of these strains were characterized by a DNA-microarray composed of oligonucleotide probes targeting ~400 resistance, adhesion and virulence associated genes.
Results: ST398 strains displayed very homogenous hybridization profiles irrespective of their host origin. This ST398 genomic profile was moderately related to that of certain human HA- or CA-lineages but distinctively lacked several virulence- and colonization-associated genes implicated in carriage in humans, such as proteases and adhesins. No enterotoxin gene was found among ST398 strains. Differences were observed in the mobile resistance gene content of ST398 strains, including antibiotic resistance determinants.
Conclusion: LA-MRSA strains represent a homogenous lineage distinct from co-local HA- and CA-MRSA strains, especially in its accessory genome content characterized by a lack of human-associated virulence and adhesion determinants. The absence of detectable enterotoxin gene among ST398 LA-MRSA strains from a wide host range is reassuring regarding their foodborne pathogenic potential.

1. Introduction

Staphylococcus aureus is a leading cause of human disease in the hospital setting as well as in the community accounting for a wide range of diseases, from superficial skin infections to bacteremia or toxic shock syndrome (Lowy, 1998). The genome sequences of several *S. aureus* strains indicate that approximately 75% of the genome, called "core" genome is conserved in all strains and composed of genes essential for growth and survival (Lindsay and Holden, 2004). This core genome is sub-divided into a so-called "core-stable" genome and a "core-variable" (CV) genome. The CV genome is composed of genes displaying different alleles which are stable within lineages and vertically transmitted. The remaining ~25%, called "accessory" genome is mostly composed of mobile genetic elements (MGEs) that differ from one strain to another and encode proteins associated with virulence and resistance (Lindsay et al., 2006).

Methicillin-resistant *S. aureus* (MRSA) is a major cause of nosocomial infections worldwide. The *mecA* gene, conferring methicillin resistance, is carried by a MGE called staphylococcal cassette chromosome *mec* (SCC*mec*). Multi-locus sequence typing of 7 housekeeping genes (MLST), combined with *SCCmec* typing, applied to a large international *S. aureus* strain collection led to the conclusion that hospital acquired MRSA (HA-MRSA) arose on multiple occasions from a relatively small number of pandemic successful MSSA lineages worldwide (Robinson and Enright, 2003).

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In Belgium, typing of MRSA strains showed that nearly 90% of strains isolated from hospitalized patients belong to four of these pandemic MRSA lineages (Denis et al., 2004, 2006). Belgium, as other countries, is also facing the emergence of MRSA infections acquired in the community (CA-MRSA). These CA-MRSA strains usually possess a distinct genetic background from HA-MRSA strains, produce the Panton–Valentine leukocidin (PVL) and present limited resistance to non-beta-lactam antibiotics (Denis et al., 2005).

MRSA may colonize or infect both pet and farm animals and transmission of MRSA can occur between animals and humans and vice versa. Usually, the strains implicated in these transmissions belong to HA-MRSA and CA-MRSA lineages (Weese et al., 2006; Strommenger et al., 2006). Recently, a peculiar MRSA genotype, resistant to digestion by *Sma*I (PFGE non typable) and belonging to MLST ST398 has been described in swine and humans in contact with swine in several countries (Huijsdens et al., 2006; Guardabassi et al., 2007; Witte et al., 2007). In Belgium, ST398 MRSA strains have been detected in a multitude of animal species and caused infection in horses, poultry, bovines and humans (Denis et al., 2009; Nemati et al., 2008, 2009; Van den Eede et al., 2009). A study in Belgian swine farms demonstrated that 70% of these hosted MRSA positive swine and that 38% of farmers and farm residents were colonized with ST398 MRSA strains (Denis et al., 2009). Human infections caused by ST398 MRSA strains have been reported as well as inter-human transmission. One outbreak was reported in a Dutch hospital. However, the nosocomial transmission rate of ST398 MRSA seems much lower than for MRSA strains belonging to other genotypes (Wulf et al., 2008a). These findings raise questions about the pathogenic potential and transmission capacities of this "livestock associated" MRSA (LA-MRSA) ST398 clone in human and animal populations.

In the present study, we explored the genetic background and the virulence and resistance gene content of HA-, CA-MRSA and ST398 LA-MRSA strains from the same country by molecular typing and microarray technique to identify biological determinants possessed by these MRSA strains that could be implicated in their epidemic and invasiveness capacity and their host specificity.

2. Materials and methods

2.1. Bacterial strains

HA-MRSA: Representative strains of the 9 major Belgian epidemic types ($n = 14$) and sporadic strains ($n = 7$) were selected from the Belgian National Reference Laboratory collection of MRSA strains from patients hospitalized in acute-care hospitals (national surveillances of 1992, 1995, 1997, 2001 and 2003 (Denis et al., 2003, 2004, 2006; Deplano et al., 2000)). The selection was based on the pulsed-field gel electrophoresis (PFGE) profiles after *Sma*I macrorestriction. Major epidemic PFGE types are types presented by at least 10% of isolates from at least one of the 5 surveillance programs. A PFGE type is considered as sporadic if it presents a pattern with a difference of more than 6 DNA fragments (Deplano et al., 2000; Tenover et al., 1995) with any other PFGE pattern in the database.

CA-MRSA: Thirteen PVL-positive strains were selected from the collection of the Belgian National Reference Laboratory for Staphylococci. This CA-MRSA collection is composed of strains that were either referred for characterization of toxin production (Denis et al., 2005) or identified as PVL positive during national surveys (Hallin et al., 2008). Representative strains of major ($n = 10$) as well as sporadic PFGE types ($n = 3$) were included.

ST398 LA-MRSA: Eighteen ST398 LA-MRSA strains were selected based on two criteria: non-typeability by PFGE after *Sma*I macrorestriction and full range of host species in the collection, including: human ($n = 9$), swine ($n = 3$), horse ($n = 2$), cattle ($n = 2$) and poultry ($n = 2$).

All strains were characterized by PCR for 16S rRNA gene, *nuc* gene, antibiotic resistance genes (*mecA*, *aac*(6′)-Ie + *aph*(2″), *ant*(4′)-Ia and *aph*(3′)-IIIa, *erm*A, *erm*C, *msr*A, *msr*B, *tet*K and *tet*M) and toxin genes (*lukS/F-PVL*, *tst*, *eta*, *etb*) as previously described (Lina et al., 1999a, 1999b; Ng et al., 2001; Vanhoof et al., 1994). Susceptibility to 13 antimicrobial agents (penicillin, erythromycin, clindamycin, ciprofloxacin, gentamicin, kanamycin, tobramycin, tetracycline, rifampin, trimethoprim-sulfamethoxazole, fusidic acid, linezolid and mupirocin) was determined by disc diffusion (Rosco Neosensitabs, Taastrup, Denmark) as previously described (Hallin et al., 2008) (Table 1).

2.2. Molecular typing methods

spa type and *agr* polymorphism were determined as previously described (Hallin et al., 2008). SCC*mec* type was determined as described by Zhang et al. (2005). MLST was performed as described by Enright et al. (2000) on ~500 bp fragments of 7 housekeeping (HK) genes. Additionally, sequences of ~450 bp fragments of 7 surface protein genes (*sas*A, *sas*B, *sas*D, *sas*E, *sas*F, *sas*H and *sas*I) were determined as described by Robinson and Enright (2003). Alleles were assigned by alignment with the *sas* gene sequences deposited in GeneBank (AY175407 to AY175464 (Robinson and Enright, 2003) and AY442690 to AY442811 (Robinson and Enright, 2004)). Sequences were aligned and concatenated; and NJ tree was built using the absolute number of nucleotide differences with the BioNumerics 5.1 software (Applied Maths, Ghent, Belgium).

2.3. Microarray analysis

The StaphVar array is a *S. aureus* 60-mer oligonucleotide DNA microarray targeting 403 virulence and resistance genes located on MGEs and allelic variants of genes located on the CV genome. The design and validation of this microarray as well as its use for characterization of CA-MRSA strains included here have been previously described (El Garch et al., 2009). Briefly, genes located on the accessory and core variable encoding toxins, adhesins, surface proteins, exoenzymes and resistance determinants of eight sequenced strains (COL, Mu50, MW2, MRSA252, MSSA476, NTCT8325, N315 and USA300) were selected. Additionally, 108 genes absent from these eight genomes but annotated with a function associated with virulence, resistance, adhesion to host cells, or encoding bacterial surface proteins and mainly located on plasmids, transposons, phages or SCCs, were selected from the literature and included.

DNA extraction was performed on strains grown on Mueller Hinton liquid medium by using DNeasy Tissue Kit following manufacturer's instructions (Qiagen, Hilden, Germany). Hybridization experiments were performed as previously described (El Garch et al., 2009) with a mix of DNA of the test strain and the reference *S. aureus* Mu50 strain (obtained from NARSA – http://www.narsa.net), labelled with red [Cy5] and green [Cy3] dyes, respectively. Slides were scanned using an Axon GenePix 4000B scanner (Molecular Devices, Sunnyvale, USA). For each slide, the images of the 408 spots, printed in triplicate, were analyzed according to the method described by Saunders et al. (2004). Briefly, individual spots were called "negative" if the signal intensity was less than 3-fold higher than the median signal intensity of negative controls. For spots presenting a signal intensity that was 3-fold higher than the median signal intensity of negative controls, the background was subtracted and the ratio "test strain/control strain intensities" was normalized to a value of one for positive controls. The spot was called "positive" if the ratio was above 0.25. When the ratio was <0.1, a spot was scored as