Comparative Exoproteome Profile of MRSA-ST9 Isolated from Pigs and Pig Handler

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Abstract MRSA-ST9-t4358 from pigs and pig handlers were analysed for exoproteins profiles. Similar protein patterns with variation in protein spot intensity were observed. No significant variation among the protein profiles indicates that transmission of MRSA-ST9 from pig to human or vice versa, do not strongly alter the gene expression patterns. Indirectly, virulence factors that acquired from pig environment might have chance to be expressed in human host.

Keywords *Staphylococcus aureus* · Exoproteome · Two-dimensional gel electrophoresis

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an established human pathogen. However, during the recent years, MRSA has been isolated from poultry and livestock [1]. Studies have shown the cross-transmission of MRSA from pig to human and causing infection [2]. The first pig-associated MRSA of sequence type 398 (MRSA-ST398) was reported in the Netherlands [3]. Thereafter, MRSA-ST398 has been marked as a pig-associated MRSA and its isolation in several countries were reported. A study conducted by us in Malaysia at 2009, a novel strain type MRSA-ST9-t4358 was isolated from pigs and pig handlers [4]. MRSA-ST9 showed evidence of cross-transmission sharing the genotype, we compare their exoproteome profiles to see, is there any difference in exoprotein profile of ST9 isolated from two different hosts.

MRSA-ST9-t4358 isolates were grown in tryptic soy broth (TSB) until exponential phase at 37 °C under orbital shaking condition (150 rpm). Subsequently, the secreted proteins (exoproteins) from supernatant were precipitated by TCA and resuspended in isoelectric focusing (IEF) buffer. For exoprotein patterns comparison, protein extracts (100 µg) were performed using analytical 2-DGE method as described by Ziebandt et al. [5]. Data were normalized using the Local Regression Model, which is recommended by PDQuest. A statistical analysis was carried out by Student's *t* test (95 % confident) to compare whether there is any difference in spot intensity between MRSA-ST9-t4358 isolated from pigs and pig handlers that are statistically significant. Preparative 2-DGE was applied prior to protein identification by MALDI-TOF/TOF. In the present study, the most dominant protein spot which was expressed in isolates from both sources were selected for protein identification.

Two hundred and seventy-two and 225 unique exoprotein spots were discriminated with high resolution on 2-DGE gels in pig isolates and pig handler isolates, respectively (Fig. 1). In the exoproteome profiles, MRSA-ST9 isolated from pigs produce 47 additional protein spots than those from pig handlers. Nine out of 47 extra protein spots were consistently found to be unique for MRSA-ST9 pig isolates. As expected, our analyses of 2-DGE gels revealed high similarity in exoprotein pattern (at least 87 %) among MRSA-ST9 pig and pig handler isolates. However, about 38.6 % (105 spots) of the total protein spots were significantly different in spot intensity. Nineteen point eighty-five percent (54 spots) were under-expressed, while 18.75 % (51 spots) over-expressed in the pig handler isolates in comparison to the pig isolates. Interestingly, eight spots with strong signal were found to be produced in all MRSA-ST9-t4358 isolates irrespective of the host of

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Fig. 1 Total 272 protein spots are separated on 2-DGE gels with 12.5 % acrylamide. The images are representing the expression of exoprotein from MRSA-ST9-t4358 pig isolates (a) or MRSA-ST9-t4358 pig handler isolates (b) that were grown to exponential phase. The arrows indicate the strongest spot among the isolates

isolation. Among these spots, the strongest spot which was excised from the gel was identified as the immunodominant staphylococcal antigen A precursor (IsaA, ISAA_STAA8). Lorenz et al. prove that IsaA has the potential value in combat the MRSA infection through the immunotherapy strategy [6].

To our knowledge, this is the first report on the basic and comparative proteome analysis of MRSA-ST9-t4358 isolated from pigs and pig handlers. The ability of *S. aureus* to produce large number of exoproteins enable it to colonize wide range of host and cause a spectrum of outcome, ranging from asymptomatic carriage to severe illness [7, 8]. The additional protein expressed in pig MRSA isolates indicates that the survival of MRSA-ST9 in pig anterior nares may require addition factors for its adaptation. The slightly difference in genetic background could be related to the not 100 % similarity among protein profile. Hecker et al. [7] reported that closely related *S. aureus* isolates would show the similar protein pattern. However, there

may be no entirely identical genetic background after a period time of interspecies or intraspecies transmission even though originated from the same clonal lineages. Thus, some spots intensity variations were found between pig and pig handler isolates despite of having same ST and *spa* types. The variation in protein spot intensity might not just rely on genome plasticity, but other factors also could contribute to this phenomenon such as translational and post-transcriptional regulation [9]. All this reflect the extraordinary capacity of this MRSA-ST9 to adapt and survive in a great variety of environment. Interestingly, the IsaA protein that was observed in this study could be an essential protein for most of the *S. aureus* as it had been described by Lorenz et al. [6]. Taken together, our results show that there is not any major difference in the exoprotein profile of the MRSA-ST9 isolated from two different hosts. Hence, the cross-transmission of MRSA-ST9 between pig and pig handler did not alter the exoprotein profile significantly. Further study should be carried out to

investigate the comprehensive proteomic profile between ST9 and ST398 to answer the question: why MRSA-ST398 cause more disease compared to MRSA-ST9?

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