Journal of Global Antimicrobial Resistance 10 (2017) 179–181

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Short Communication

Spa typing and identification of *pvl* genes of meticillin-resistant *Staphylococcus aureus* isolated from a Libyan hospital in Tripoli☆

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ARTICLE INFO

Article history:
Received 20 April 2017
Received in revised form 17 May 2017
Accepted 2 June 2017
Available online 19 July 2017

Keywords:
Community-acquired MRSA
spa typing
pvl genes
Libya

ABSTRACT

Objectives: The purpose of the study was to investigate the molecular characteristics of meticillin-resistant *Staphylococcus aureus* (MRSA) isolated from clinical sources in Tripoli, Libya.
Methods: A total of 95 MRSA strains collected at the Tripoli medical Centre were investigated by *spa* typing and identification of the Panton–Valentine Leukocidin (*pvl*) genes.
Results: A total of 26 *spa* types were characterized and distributed among nine clonal complexes; CC5 (n = 32), CC80 (n = 18), CC8 (n = 17) and CC22 (n = 12) were the most prevalent clonal complexes. In total, 34% of the isolates were positive for PVL.
Conclusions: This study demonstrated the presence of CA-MRSA and *pvl* positive strains in hospital settings and underlines the importance of using molecular typing to investigate the epidemiology of MRSA. Preventative measures and surveillance systems are needed to control and minimize the spread of MRSA in the Libyan health care system.

1. Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen as well as a major global public health concern [1,2]. Since the late 1990s, a remarkable epidemiological change occurred – the emergence of lineages of community-associated (CA)-MRSA – and correlated with distinctive changes in MRSA geographical distributions [3,4]. This is attributed largely to complex dynamics between healthcare settings and communities presenting more significant challenges for MRSA management and infection control, both at regional and international levels [5]. As a result, the previously established molecular and epidemiological features (i.e. Panton–Valentine leukocidin -PVL coded by the lukS and lukF genes and antimicrobial susceptibility profile) used to distinguish healthcare associated (HA)-MRSA from CA-MRSA are no longer commonly used [3].

Recent reports and large scale multi-national investigations have concluded that CA-MRSA clones found in North Africa, Europe and the Middle-east likely originated from the sub-Saharan African region through migration and population movements [4,6]. In Africa, *S. aureus* has not been fully elucidated but its epidemiology is recognized as unique, likely due to the continent's diverse geographical, environmental, and cultural aspects [7,8]. Over the past decades, other studies have reported the occurrence of MRSA from Libya, largely from/at hospital settings; however, epidemiological and molecular data are limited and not applicable to understanding modern trends [9,10]. Here, we investigated MRSA strains isolated and collected from the largest Libyan hospital in Tripoli, using *spa* typing and the identification of the Panton–Valentine Leukocidin (*pvl*) genes. The Tripoli medical centre is the largest Libyan hospital by size and population, providing various health care services to the capital as well the north-west areas of Libya.

☆ The work was done at Tripoli Medical Centre and Faculty of Veterinary Medicine, Tripoli, Libya, and Statens Serum Institut, Copenhagen, Denmark.

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http://dx.doi.org/10.1016/j.jgar.2017.06.004

2. Materials and methods

2.1. Bacterial strains

A total of 95 MRSA strains were collected throughout the years 2008 (n = 40) and 2014 (n = 55). The strains were originally isolated from clinical samples of admitted and hospitalized patients and stored throughout the relevant years. The MRSA strains originated from 45 females and 44 males (six isolates with no information on gender) and collected from different clinical samples, mainly swabs from nose, ears, wounds sputum, throat, pus, and urine. Samples were initially processed using standard laboratory techniques and identified as *S. aureus* from non-selective medium based on Gram-positive cocci in clusters, catalase positive and coagulase test. Isolated *S. aureus* colonies were further confirmed and tested for antimicrobial susceptibility using VITEK (VITEK-Compact 2, BioMérieux) automated identification systems. Strains were characterized by multidrug resistance to different antimicrobial classes with variable resistance profiles but fully susceptibility to glycopeptides, linezolid, and daptomycin. Confirmed MRSA were stored at −80 °C.

2.2. Molecular investigations (mec & pvl gene and spa typing)

Isolates were additionally confirmed as MRSA by a multiplex PCR detecting the *mecA*,*spa* and *pvl* genes [11]. The *spa* PCR product was sequenced at Beckman Coulter Genomics (Takeley, Essex, UK). The *spa* types were determined using BioNumerics v6.6 (Applied Maths, Sint-Martens-Latem, Belgium). The *spa* types were assigned to the corresponding Multi Locus Sequence Typing (MLST) clonal complexes (CCs) based on the *spa* types' association to known sequence types (ST) as well as the ST's relations to CCs by using the Ridom database (www.ridom.de) and eBurst v.3 (www.mlst.net). The *spa* types without known MLST associations were placed in a CC if the difference in repeat patterns of a *spa* type with known MLST associations could have occurred by no more than two genetic events.

3. Results

A total of 26 *spa* types were characterized and distributed in nine clonal complexes; three *spa* types (t640, t1366, and t4158) could not be assigned to any CC. The most prevalent *spa* types were t044/CC80, t002/CC5, t005/CC22 and t037/CC8 (Table 1). Five isolates demonstrated an unusual repeat pattern of a *spa* type since the fourth repeat contained 25 base pairs, in contrast to 24 base pairs (or 21, 27 and 30 base pairs) and thus could not be assigned to a known *spa* type. The additional base pair was an extra adenin inserted in a stretch of six adenins. In all other respects, the repeat pattern was identical to repeat 02. The other repeats were identical to the repeat succession of t037 (15-12-16-02-25-17-24) and thus assigned the *spa* type as variant-t037. One new *spa* type, t14971 (repeat succession 09-02-16-34-13-17-34-13), was identified in an isolate from 2008. There were variations of the distribution of *spa* types for at least two distinct years, since 22 *spa* types were unique between sampling years (10 unique in 2008, 12 unique in 2014, Table 1). CC5 (n = 32), CC80 (n = 18), CC8 (n = 17) and CC22 (n = 12) were the most prevalent clonal complexes, comprising 83% of the isolates. In total, 34% of the isolates were positive for PVL. All t044/CC80 and t005/CC22 were positive for PVL as well as three additional *spa* types (Table 1).

Table 1
spa types and frequency of PVL gene by year among MRSA isolates.

spa type/CC	Year		Total	PVL positive (%)
	2008	2014		
t044/CC80	9	8	17	17 (100)
t002/CC5	13	3	16	0 (0)
t005/CC22	1	10	11	11 (100)
t037/CC8		10	10	0 (0)
Variant-t037/CC8		5	5	0 (0)
t242/CC5	5		5	0 (0)
t267/CC97	1	3	4	0 (0)
t127/CC1	1	2	3	2 (67)
t1230/CC8		2	2	0 (0)
t3702/CC5	2		2	0 (0)
t3778/CC5		2	2	0 (0)
t084/CC15		2	2	0 (0)
t223/CC5		2	2	0 (0)
t852/CC22		1	1	1 (100)
t640/UNK	1		1	0 (0)
t4173/CC97		1	1	0 (0)
t1081/CC45	1		1	0 (0)
t010/CC5	1		1	0 (0)
t1366/UNK	1		1	0 (0)
t4158/UNK		1	1	0 (0)
t311/CC5		1	1	0 (0)
t509/CC5		1	1	0 (0)
t318/CC30	1		1	0 (0)
t688/CC5	1		1	0 (0)
t14971/CC45	1		1	0 (0)
t376/CC80	1		1	1 (100)
t2249/CC5		1	1	0 (0)
Total	40	55	95	32 (34)

UNK = unknown association to CC.

4. Discussion

Little is understood about the molecular epidemiology of MRSA in Libya. Genotyping and molecular methods such as *pvl*-PCR, MLST and *spa* typing are useful and essential approaches to investigate the epidemiology of MRSA [12]. Our study concurs with other similar findings related to the widely documented strains of MRSA from this region as well as Europe [4,10,13–16]. For instance, BenDarif et al. [13] have recently reported t002/CC5, CC80 (no *spa* type indicated) and t022/CC22 as the most prevalent among 202 studied isolates. However, in our study we did not find any t022/CC22 even though CC22 was among the most common clonal complex. This suggests that care is needed with general interpretations of results based on non-systematic collection of isolates, as represented both with the present and former study.

The high prevalence of CC80 strains in this study was not unexpected. The CC80 has previously been described from North African countries [14–16]. This is also the major European community-acquired MRSA (CA-MRSA) clone which was previously described as originating from a meticillin-sensitive *S. aureus* from sub-Saharan countries [4]. After a single introduction of a *Staphylococcus* Chromosome Cassette containing the *mecA* gene (SCCmec), this lineage spread successfully to Northern Africa, the Middle East and Europe.

In a previous study, *pvl* gene prevalence was 29% amongst inpatient children, mothers of inpatient children, healthcare workers, and outpatient children in Tripoli, Libya [10]. In our study, the distribution of *pvl* genes was highly related to clonal complexes. For example, *pvl* genes were found in all isolates belonging to CC22 and CC80 and in two out of three CC1. The role of PVL has been much debated. A recent study indicated that the presence of *pvl* genes in MRSA impacted the severity of disease and clinical outcome in patients with hospital acquired pneumonia due to MRSA [17]. Another study demonstrated an increased risk of death associated with PVL positive *S. aureus* blood-stream infections [18]. None of the PVL positive samples in this study were from invasive infections but the introduction of PVL positive clones into hospital settings is worrisome.

The finding of CA-MRSA clones in a hospital setting underlines the blurred distinction between HA-MRSA clones and CA-MRSA [12]. Despite the paucity of information available about the studied isolates, particularly in relation to patients and thus uncertain regarding nosocomial transmission versus prior acquisition, the introduction of successful CA-MRSA clones into hospitals warrants attention. Previously, a carriage rate of MRSA at 19% among doctors and nurses in Tripoli was reported [9]. Entrance into hospital settings could be minimized by screening upon admission as well as transmission limited within the hospital by strict adherence to control procedures.

The major limitation of this study is that bacterial strains are not collected in a systematic or consecutive manner and also do not allow for a later retrospective look at patient data. The limitation is shared with another recently published study from Libya [13]. However, this study provides additional insight into the distribution of MRSA clones in Libya and highlights the importance of molecular surveillance of MRSA and other nosocomial pathogens.

In conclusion, this study demonstrated the presence of CA-MRSA strains in a major Libyan hospital. This study also indicates the urgent need for strict control measures and advanced surveillance screening systems that involve both healthcare and the community settings to minimize spread.

Funding

No funding.

Ethical approval

Not required.

Competing interests

None.

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