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Original Article

Methicillin-resistant *Staphylococcus aureus* with *Panton-Valentine leukocidin* gene from a pediatric hospital in Morocco

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Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial and community infections. These infections are becoming increasingly difficult to combat, because of emerging resistance to all classes of antibiotics. Panton-Valentine leukocidin (PVL) is an important virulence factor in MRSA and causes white blood cell destruction, necrosis, and accelerated apoptosis. The aim of this study was to determine the frequency of *pvl*-positive MRSA in a pediatric hospital, in Marrakech, Morocco.

Methodology: 53 isolates of MRSA were recovered in the hospital from December 2010 to May 2014, and confirmed with biochemical tests (coagulase, mannitol fermentation, and DNase). Then, polymerase chain reaction (PCR) was used to detect *pvl*.

Results: Among the 259 *Staphylococcus aureus* isolates collected from various clinical specimens, 53 were identified as MRSA; and the presence of the *PVL* gene was investigated in them using PCR analysis. Out of the 53 MRSA isolates, only 1 (1.89%) was positive for *pvl* This *pvl*-positive MRSA isolate was characterized as staphylococcal cassette chromosome mec IV (SCCmec IV), a type commonly associated with community-acquired MRSA infections.

Conclusions: The study revealed a relatively low prevalence of *PVL*-positive MRSA among pediatric patients at the University Hospital Center CHU Mohamed VI in Marrakech, with only 1.89% of MRSA isolates testing positive for *pvl*. Despite this low prevalence, the presence of *PVL*-positive strains accentuates a potential risk for severe infections in vulnerable children. These findings underscore the imperative need for sustained surveillance and rigorous infection control measures to mitigate the spread of MRSA and other resistant pathogens.

Key words: Panton-Valentine leukocidin; *pvl*; methicillin resistant *Staphylococcus aureus*.

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Introduction

Staphylococcus aureus (*S. aureus*) is a significant bacterium, commonly found as part of the normal human microflora. Approximately 20–30% of the global population harbors *S. aureus* asymptomatically [1]. While typically benign, *S. aureus* can cause a spectrum of infections ranging from mild skin and soft tissue infections to severe systemic conditions with multiorgan involvement.

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) represents a major public health challenge due to its resistance to methicillin and nearly all β-lactam antibiotics. MRSA was first identified shortly after the introduction of methicillin in 1961 [2]. MRSA was initially recognized as a nosocomial pathogen, but has increasingly been reported as a community-acquired infection [3]. MRSA strains exhibit resistance to both penicillins and cephalosporins and are distributed globally, highlighting a critical issue in both hospital and community settings [4].

Clinically, MRSA infections are of particular concern due to their association with severe disease outcomes. Infections caused by MRSA can range from minor skin lesions to life-threatening conditions such as necrotizing pneumonia [5,6]. A key virulence factor in some MRSA strains is the Panton-Valentine leukocidin (PVL), a toxin that exacerbates the severity of infections by targeting and destroying host leukocytes [7]. PVL contributes to the pathogenesis of MRSA by causing significant tissue damage and immune system disruption, leading to more severe clinical manifestations [8].

On a molecular level, MRSA strains possess a unique penicillin binding protein 2a (PBP2a), which has a markedly reduced affinity for β-lactam antibiotics, underpinning their resistance [7]. PVL is one of several exoproteins produced by MRSA that enhances its virulence. Understanding the molecular characteristics

of MRSA, including the presence of PVL and the type of staphylococcal cassette chromosome mec (SCCmec), is essential for comprehending the bacterium's full pathogenic potential and resistance profile [9].

Despite extensive research on MRSA and PVL in various regions, there is limited data on the prevalence of PVL-positive MRSA in Morocco. This study aimed to address this gap by evaluating the prevalence of the *pvl* gene in MRSA isolates obtained from pediatric patients at a regional pediatric hospital in Marrakech. Additionally, the study aimed to correlate the presence of the *pvl* gene with SCCmec types to provide insights into the local epidemiological landscape. This information will be critical for informing antimicrobial therapy and infection control practices in the region.

Methodology

Bacterial strains

This study was conducted from December 2010 to May 2014 at a pediatric hospital, CHU Mohamed VI, Marrakech, Morocco. The samples were collected from children of age ranging from 2 days to 15 years. Out of 259 isolates of *S. aureus* that were collected, 53 (20.46%) were found to be methicillin resistant; out of these, 75.47% were from blood samples, 11.32% were from catheter, and 13.21% were from other specimens. The average age of the children from whom the samples were collected was 24 months; 54.72% of samples were isolated from male patients, and 45.28% were collected from female patients. Among the isolates, 53 were identified as MRSA, and the presence of the *pvl* gene was assessed by polymerase chain reaction (PCR).

Isolates identification

All isolates were previously identified by classic microbiological methods including colony morphology, mannitol fermentation, Gram staining, catalase test, coagulase test, and the API Staph test. Methicillin resistance was confirmed using a cefoxitin disk (30 μg) on Mueller-Hinton agar plates (Bio-Rad, Marnes-la Coquette, France), as recommended by the French Society for Microbiology (FSM, 2013) [10], and PCR was used to confirm the presence of *mecA* and *nuc* genes.

DNA extraction

Genomic DNA of the MRSA strains were extracted by using a standard phenol–chloroform procedure, as described elsewhere [11].

pvl gene detection

The *pvl* gene was detected by PCR amplification following the protocols that were previously defined [12,13]. The PCR products were mixed with 1 μL loading buffer and separated by 1.5% agarose gel electrophoresis at 75 V for 90 minutes. The gel was stained with ethidium bromide for 15 minutes and observed under the UV trans-illuminator. (Bio-Rad, Marnes-la Coquette, France). The positive control strain used for *pvl* detection was HT2003 0642.

Results

Bacterial isolates

Out of 259 isolates of *S. aureus*, 53 (20.46 %) were methicillin resistant.

PCR detection of pvl in Moroccan strains

The *pvl* gene was identified as a 433 bp band in the PCR-amplified product, using a DNA molecular marker (100 bp ladder). The *pvl* gene was detected in only one isolate out of the 53 MRSA isolates included in the study (1.88%). The clinical sources of *pvl* were respiratory specimens (1.88%). No other association between antibiotic resistance and presence of *pvl* was found. Only one isolate was positive in our study Table 1.

Discussion

The pathogenicity of *S. aureus* depends on various bacterial surface components and extracellular proteins. However, the precise role of single virulence determinants in relation to infection is difficult to establish. The frequent recovery of staphylococcal isolates that produce leukocidal toxins from patients with deep skin and soft tissue infections; particularly furunculosis, cutaneous abscesses, and severe necrotizing pneumonia, suggests that the PVL is a virulence factor that has a major role in pathogenicity [14].

In 1932, Panton and Valentine described PVL as a virulence factor belonging to the family of synergohy-

Table 1. Distribution of *plv* in clinical isolates of MRSA based on the biological source and service department.

Source of samples	MRSA/*plv* (%)	Source department	MRSA /*plv* (%)
Blood culture	(75.47/0 %)	Pediatrics	(37.73/0 %)
Catheters	(11.32%/0%)	Neonatology	(33.96 /0 %)
Respiratory specimen	(5. 66/1.88%)	Intensive care pediatric	(16,.98/1.88%)
Pus/cerebrospinal fluid/biological	(5.66/0%)	Surgery Pediatric	(11.32 /0%)

MRSA: methicillin-resistant *Staphylococcus aureus.*

menotropic toxins [15]. These toxins form pores in the membrane of the host defense cells by synergistic action of 2 secretory proteins, designated LukS-PV and LukF-PV, which are encoded by 2 co-transcribed genes of a prophage integrated in the *S. aureus* chromosome [16]. PVL may be produced by different strains of *S. aureus*, in particular both methicillin-sensitive *S. aureus* (MSSA) and MRSA [17].

Both colonizing and pathogenic potentials of *S. aureus* are related to the virulence factors carried by the circulating strains [18]. A high prevalence of *pvl* has been documented in isolates of MRSA [19]. PVL is a pore- forming leukotoxin composed of two components — S and F — which are encoded by the *LukS-PV* and *LukF-PV* genes in the lysogenic phage phiSLT [20]. Due to the epidemiological association between *PVL* and community acquired MRSA (CA-MRSA) isolates, many efforts have been directed toward identifying the pathogenic role of this toxin; but the results have been controversial. Several in vitro studies have shown that PVL induces cell lysis by forming pores in the membranes of polymorphonuclear (PMN) cells, and it induces apoptosis by interacting with the mitochondrial membrane [21], and activating downstream TLR-2 signaling pathways, leading to an inflammatory response [22], and complement receptor-mediated cytotoxicity [23]. However, animal model studies have failed to demonstrate a pathogenic role for *pvl* in staphylococcal infections [24]. Despite this discrepancy, *pvl* has been increasingly associated with severe clinical manifestations of *S. aureus* infections. Thus, more research is needed to identify subjects at risk of developing severe forms of infection [25]. *pvl* is mostly associated with CA-MRSA infections and distinguishable from nosocomial MRSA by non-multidrug resistance and carriage of the SCCmec type IV [26]. Other researchers who detected *pvl* in *S. aureus* using immunodiffusion agar in a hospital in France, reported that PVL producing *S. aureus* were responsible mostly for necrotizing skin infections such as furuncle and abscess [27]. It is noted in various reports that a patient with abscess or recurrent furuncle should be primarily suspected of PVL related *S. aureus* infection [28]. This is especially true in high-risk groups such as athletes with close encounter sports.

In this study, *pvl* was detected using PCR and analyzed by electrophoresis on a 1.5% agar gel. It has also been noted in various studies that *S. aureus* carrying *pvl* are the cause of epidemic infections and have been referred to as "super adapted *S. aureus* isolates" [29]. However, some studies have associated *S. aureus* carrying *pvl* to MRSA and especially CA-MRSA [30]. These observations are in agreement with results from Wannet *et al*. in Holland [30]. The first MRSA isolates positive for *pvl* were identified in the late 1990s [31]. In recent years, these strains have spread globally [32].

In this study, 53 MRSA isolates were collected from a hospital in Morocco. PCR tests showed that 1.88% of the isolates carried *pvl*. This finding is in agreement with previously reported low *pvl* prevalence by Prevost *et al.* (1.4% in 100 carriage isolates), and Von Eiff *et al.* (1.4% in 210 carriage isolates) [16,33]. However, a higher prevalence of *pvl* (38.9%) was reported in *S. aureus* strains causing abscesses and arthritis [26]. This finding is also in agreement with the proposed involvement of *pvl* in severe and invasive (soft tissue) staphylococcal infections [34]. Most studies have found a strong epidemiological association between *pvl* and infections caused by MRSA. In Colombia, the reported prevalence of *pvl* in MRSA isolates ranged from 73 to 98.7%. Jimenez *et al.* found that 73% of MRSA isolates obtained from pediatric infections in Medellin were positive for *pvl* [35].

Meanwhile, it was reported that 88% of MRSA isolates obtained from patients of the same age in the city of Bucaramanga were positive for *pvl*. In Bogota, Marquez-Ortiz *et al.* determined that 98.7% of the MRSA isolates that caused pediatric infections were positive for *pvl* [36]. One plausible explanation for this lower frequency than that reported by our study is that our study population was smaller, and we focused only on nosocomial hospital-associated MRSA (HA-MRSA) isolates. There are reports of *pvl* MRSA frequencies < 1% in northern Spain [37]; < 20% in Turkey [38], Greece [39] and Lebanon [40]; and > 40% in India [41], Nepal [42] and Africa [43]. The prevalence of *pvl* MRSA in these countries is much higher than that reported from European countries [14]. In a recent multicenter prospective European study, the prevalence of *pvl S. aureus* was reported to be 18.6, and 7.8% of the isolates were MRSA [44]. Ritz *et al.* demonstrated that the proportion of *pvl S. aureus* was higher in infections caused by MRSA (74–100%) than those caused by MSSA (9–46%). The proportion was dependent on the prevalence of MRSA in the respective regions [45]. The *pvl* positivity rate was 77–100% in CA-MRSA infections, while it is less than 4% in HA-MRSA infections [46].

In general, the results of this study demonstrate low prevalence of MRSA carrying *pvl* in the hospital from which the isolates were collected. These isolates were resistant to multiple antibiotics. As has been frequently reported, MRSAs are bacteria resistant to a series of

antibiotics in addition to methicillin [47]. In one study on MRSA in Algeria, *pvl* was the most common toxin producing gene identified [48]. One of the most important determinants of the severity and outcome of any infection is the presence of virulence factors in the infectious agent. Multiple virulence factors have been associated with the pathogenic potential of *S. aureus* [19].

PVL is the most studied pathogenic factor in *S aureus*. There is historical, epidemiological, and biochemical evidence that supports the implication of this toxin in the pathogenesis of *S. aureus*; however, it is unclear whether *pvl* affects the presentation or severity of infection [49]. The role of *pvl* in severe MRSA infections is debated due to conflicting data from epidemiological studies, in vitro cell culture experiments, and different animal disease models [24,50]. *pvl* was found in almost all MRSA strains that caused CA-MRSA infections, such as necrotizing pneumonia, and skin and soft tissue infections; therefore, it was assumed to be a crucial virulence factor. These diseases are characterized by large tissue necrosis and leukopenia, which have been linked to the ability of *pvl* to kill neutrophils, the primary defending cells against invading bacteria. Since PVL virulence factor is carried by a bacteriophage and is also transferable to other *S. aureus* [51], the risk of epidemic infection with such isolates in hospitals is high. Physicians should thus adopt suitable strategies to identify such isolates and assign quick and suitable treatments. It is therefore very important to identify and decolonize the carriers because infections by these isolates are very invasive, and even lethal, and their epidemics will impose irremediable outcomes.

Conclusions

MRSA is an important nosocomial pathogen present in hospitals in Morocco. We detected a frequency of 1.88% *pvl* positivity in MRSA isolates. Therapeutic strategies should include agents that can inhibit this virulence factor. In addition, a warning should be considered in patient care and prevention policies to address the broader risks associated with *S. aureus* infections.

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Conflict of interests

No conflict of interests is declared.

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