

REVIEW ARTICLE

Community acquired pneumonia due to Panton-Valentine leukocidin producing methicillin resistant *Staphylococcus aureus* in Greece.

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Abstract

Community acquired pneumonia due to methicillin resistant *Staphylococcus aureus* (MRSA) is an uncommon but severe infection that affects predominantly young healthy subjects and confers a high mortality risk. Most community acquired MRSA isolates produce the Panton-Valentine leukocidin (PVL) which plays a major role in their pathogenicity. In Greece, an increasing percentage of community acquired *Staphylococci* are methicillin resistant. A single clone, SCCmec IV - ST80, has emerged as dominant, expanding in the community, infiltrating the hospital setting and replacing older clones. It is almost universally PVL positive while demonstrating significant rates of resistance to several first line antibiotics. As a country with intense population mobility due to tourism and immigration, the possible export of such problematic strains is worrisome. Review of eight published case reports of community acquired MRSA pneumonia in Greece display the dramatic morbidity and mortality associated with this infection and underline the need for early and appropriate antibiotic treatment as well as ICU support.

Key-words: *Staphylococcus aureus*, Community acquired pneumonia, Methicillin resistant, Greece

1. Introduction

Staphylococcus aureus is a bacterial species able to cause a broad variety of infections ranging from minor skin infections to severe pneumonia, bacteremia and lethal shock. Methicillin Resistant *Staphylococcus aureus* (MRSA) has already a presence of several decades; it appeared as soon as two years after the introduction of methicillin which in turn was manufactured in order to overcome resistance to penicillin by the relevant enzymes¹. MRSA was initially confirmed as a nosocomial pathogen causing skin and soft tissue infections, pneumonia, endocarditis, osteomyelitis and foreign body infections. Meanwhile MRSA percentage was alarmingly increasing and spreading worldwide^{2,3}. On top of that MRSA appeared in the community already in the 1980s. Most of these cases could be linked to healthcare settings⁴, yet in late 1990s a significant epidemiological evolution occurred with the emergence of “genuine” community acquired MRSA infections in hitherto healthy young subjects⁵. Throughout the next years CA-MRSA appeared in multiple areas of the world and spread widely representing a significant public health threat⁶.

1.1 Community versus Hospital Acquired MRSA

Resistance to methicillin is considered a major distinctive characteristic of *Staphylococcus* such that MRSA strains are clinically approached as “subspecies”. A further clinical subdivision is between CA-MRSA and HA-MRSA since they present discrete and often contrasting features. In fact HA-MRSA and CA-MRSA are genetically distinct; methicillin resistance is conferred by the *mec* gene, itself part of a mobile chromosomal element called the Staphylococcal Cassette Chromosome (SCCmec). HA-MRSA contains the SCCmec

genetic element of type I, II, or III, while CA-MRSA contains a SCCmec element of type IV and less often V (while types up to XI have been until now added)³. From a clinical perspective, HA-MRSA is a hospital pathogen mostly infecting vulnerable patients while CA-MRSA may affect previously healthy young adults in the community. Most important however in clinical practice is the difference in drug sensitivities, with CA-MRSA being usually susceptible to most non β -lactam antibiotics, while HA-MRSA expressing multi-drug resistance.

1.2 CA-MRSA Pneumonia

CA-MRSA is an unusual cause of Community-acquired pneumonia, responsible for up to 0.5% of cases. The overall incidence of CA-MRSA pneumonia is estimated at 0.51-0.64 per 100000 population⁷. However, it is a dire infection with 75-85% of patients reaching the ICU and 20-60% succumbing to the disease⁸. The severity of the statistics is further emphasized by the patients affected; young previously healthy adults in up to 75% of cases⁹. A preceding influenza, either documented or not, is characteristic. Skin trauma, skin to skin contact and exposure to contaminated material are risk factors for MRSA colonization and define risk groups for CA-MRSA infection such as athletes, injection drug users, military personnel etc. Traditional risk factors for Staphylococcal infection must be also taken into account: recent hospitalization, surgery, dialysis, residence in a long-term care facility in the last year, invasive devices, and history of MRSA infection or colonization⁹.

CA-MRSA pneumonia presents with rapidly evolving septic syndrome and respiratory failure. There is high fever, dyspnea and respiratory distress, hemoptysis, tachycardia

and hypotension; multi-lobar consolidation, typically with cavitation, pleural effusions and airway hemorrhage; these are accompanied by leukopenia and markedly elevated C-reactive protein⁹. These features, more than any eventual risk factors, should prompt the upgrading of the antibiotic scheme since the recommended initial empirical treatment of CAP may not provide adequate coverage for MRSA.

1.3 Panton-Valentine leukocidin

Staphylococci may produce a variety of virulent factors. The best known and possibly the most important is the Panton-Valentine leukocidin (PVL). PVL is a cytotoxin that causes leukocyte destruction and tissue necrosis¹⁰. Although its precise role and interaction with other toxins is not fully understood, PVL has a clear role in the pathogenesis of necrotizing pneumonia by CA-MRSA. It is encoded by two genes, namely lukS-PV and lukF-PV which are present in the majority of CA-MRSA strains but not confined to them; PVL may be produced by HA-MRSA and even by up to 12% of MSSA strains¹¹. It is noteworthy that PVL positive Staphylococcal pneumonia appears of equal severity whether due to MRSA or MSSA strains¹².

1.4 CA-MRSA pneumonia treatment

Empirical treatment should be initiated immediately in patients suspected of MRSA infection, particularly in cases of fulminant community-acquired pneumonia. Combinations of vancomycin, linezolid, clindamycin and rifampicin have been reported to successfully treat PVL-MRSA pneumonia. CA-MRSA is usually susceptible to most non-lactam antibiotics, in contrast to HA-MRSA which is not only by definition resistant to β -lactams but additionally to a wide range of other antibiotic

classes. The use of linezolid, clindamycin and rifampicin, often in combination, is supported as able to reduce the production of PVL toxin, a therapeutic target not met by the glycopeptides^{13,14}. Polyvalent intravenous immunoglobulin (IVIg) may represent a valuable adjunct to antibiotic therapy aiming to inhibit the detrimental effects of the PVL toxin¹⁵.

2. Community Acquired MRSA pneumonia in Greece - Review of Case Reports

Eight cases of CA-MRSA pneumonia in adult patients have been recorded in Greece¹⁶⁻²¹; one further case with similar clinical features was due to CA-MSSA²¹. All cases, including the one caused by an MSSA, were PVL-positive. Six isolates were classified as SCCmec IV - ST80 whereas two isolates were SCCmec V. All patients but one presented with septic shock and respiratory insufficiency and were treated with mechanical ventilation in the ICU; five out of eight patients recovered after prolonged hospitalization (mean ICU stay 35.5 days) while the remaining three succumbed to the disease within 1-3 days after ICU admission. Mean patients' age was 42.75 (17 to 74 years old), and half were men. Two patients had a recent skin infection that had subsided and was compatible but not confirmed as Staphylococcal. Another female patient had Ovarian Hyperstimulation Syndrome after IVF. The remaining patients had an unremarkable medical history and no one had any risk factor for Staphylococcal or MRSA in particular infection. There was a flu-like prodrome in two patients but influenza was not documented in any case. Markedly increased inflammation markers were present in all patients while leukopenia was recorded in four cases. Parenchymal necrosis in the form of either small or large cavities were universally present on CT imaging. Pulmonary hemorrhage was

reported in four cases. Pleural complications were common. There were pneumothoraces in three patients and pleural empyemas treated by drainage in three patients. All patients were treated with a combination of antistaphylococcal drugs. In one patient IVIG was added, apparently contributing to a favourable outcome. Antibiotic sensitivities data were not explicitly reported. Data provided for 7/8 CA-MRSA isolates demonstrated sensitivity to most drugs with no resistance encountered to vancomycin, linezolid, clindamycin and rifampicin. Resistance to tetracycline, fusidic acid, macrolides and aminoglycosides was reported in more than two instances each. Resistance to quinolones was implied in one case¹⁹.

3. Discussion

Studies on CA-MRSA epidemiology in Greece have not focused on cases of pneumonia. However, herein reported cases affirmed the predominance of the SCCmecIV ST 80 clone recovered in 6 out of 8 patients described. All 8 cases were PVL positive underlining the pathogenic role of the PVL toxin. A further MSSA PVL positive case²¹ was of equal severity and clinical features, in accordance with published evidence^{12, 22}. The severity of the infection was also depicted with 3/8 patients (37.5%) succumbing to the disease and all but one (87.5%) admitted to the ICU. Nevertheless, since the number of cases is small and confined to case reports we cannot exclude the possibility that milder cases might be underreported.

CA-MRSA constitutes a rare occurrence among community acquired pneumonias. Among clinical features that may point to a CA-MRSA aetiology and direct the clinician in adapting antimicrobial treatment, parenchymal necrosis was universally present in all 8 reported cases.

Leucopenia, sepsis and septic shock, pulmonary hemorrhage and pleural complications were also common and were suggestive of MRSA aetiology. In contrast, definitive risk factors for Staphylococcal in general or MRSA in particular infection were not present.

Antibiotic sensitivities were better than expected in the reviewed cases, given the high rates of antibiotic drug resistances in Greece. Besides universal susceptibility to linezolid, vancomycin and rifampin there was no resistance either to clindamycin, an important antistaphylococcal agent. Furthermore, resistance to quinolones was present in only 1/7 isolates in spite of their widespread use in Greece in the treatment of community acquired pneumonia - among other indications. However, even the administration of a combination of active antibiotics cannot guarantee clinical success particularly once septic shock and multiorgan dysfunction occur. The number of cases reviewed is too small to draw conclusions on the appropriate antibiotic scheme. In all cases initial treatment for CA pneumonia was clinically and microbiologically inadequate and had to be escalated and adapted. Early and appropriate treatment, combination of agents that inhibit toxin synthesis and systematic support -ideally in the ICU- appear to be of critical importance.

3.1 CA-MRSA in Greece - epidemiology

The first PVL-positive HA-MRSA isolate in Greece was reported in 1998²³. An increase in the incidence of PVL-positive CA-MRSA and HA-MRSA infections followed. A 3-year survey (2001-2003) examining the spread of PVL-positive SA in Greece collected a total of 1058 isolates²⁴. CA isolates concerned skin and soft tissue infections whereas HA isolates were associated with surgical wound or

prosthetic devices' infections. An increase in the percentage of PVL-positive MRSA isolates, both CA and HA was recorded. 45% of MRSA were PVL-positive, compared to 12% for MSSA. 72% of the CA-MRSA isolates and 23% of the HA-MRSA isolates were PVL-positive. In contrast, only 20% of CA-MSSA and 5% of HA-MSSA were PVL-positive. Further microbiological analysis revealed that all PVL-positive MRSA isolates belonged to a single clone - SCCmec type IV, sequence type ST80 - that was disseminated in the community and the hospital.

The rapid propagation and dominance of this specific clone, already prevalent in Europe was confirmed in a subsequent study. A microbiological survey carried out between 2006 and 2007 examined SA from adults with CA skin and soft tissue infections²⁵. Among 88 Staphylococcal infections, 27(30.7%) were found to be MRSA; all of them belonged to SCCmec IV - ST80 clone and carried the PVL producing genes.

The evolution of MRSA epidemiology was nicely demonstrated in a study examining MRSA carriage on consecutive patients admitted to a surgical unit of a tertiary care hospital in Athens²⁶. Out of 925 patients, 51 (5.5%) were MRSA positive; 15 of 51 cases fulfilled epidemiologic criteria for CA-MRSA, yet 6/15 corresponded to HA-MRSA clones. Of the CA-MRSA clones 7/9 were ST80-IV and 2/9 ST30-IV. In parallel, among 36 epidemiologically HA-MRSA cases, 7/36 were in fact due to CA-MRSA clones and in particular the ST80-IV. All 14 ST80-IV clones were PVL positive whereas in only one more case PVL positivity was present, an ST30-IV clone. These data highlight the blurring of the epidemiological distinction between

community and hospital acquired MRSA infection with evident clinical consequences since 3/14 PVL positive MRSA clones exhibited multiple antibiotic resistance.

A most recent study affirmed the preponderance of the SCCmec IV ST-80 clone but revealed a more varied microbiology with several other CA-MRSA clones. A large 12-year survey between 2001 and 2012 in 6 tertiary hospitals in Greece analyzed 4614 MRSA isolates²⁷. The majority (72.5%) were CA-MRSA causing skin and soft tissue infections. A small but not negligible percentage of 2.4% was recovered from bacteremias while another 1.4% from respiratory tract infections. HA-MRSA isolates had more varied target of infection. Respiratory tract infections corresponded to 6.9%. Interestingly, the percentage of MRSA isolates showed a gradual increase from 2001 to 2008 with a subtle decline thereafter. The frequency of the "European clone" SCCmec IV - ST80 increased yearly from 28.6% to 73.3%, gradually replacing other clones, initially in the community, and subsequently infiltrating the hospital setting. In total SCCmec IV-ST80 clone comprised 75.4% of CA-MRSA isolates and was almost universally PVL-positive (96.7%). Other main clones in this study were the ST239-III (10.3% of CA-MRSA but the main HA-MRSA clone ie 55.5%) , ST30-IV (9.5%), ST377-V (1.4%) and ST225-II (0.2%). PVL positivity was unusual in these clones, ranging between 0.2% and 4%.

3.2 CA-MRSA in Greece - antibiotic sensitivities

Antimicrobial drug resistance rates in Greece are among the highest in Europe²⁸. High unrestricted antibiotic consumption both in hospital and in the community combined with

insufficient infrastructure and resources for infection control are responsible for this unflattering performance.

Two studies examined in particular the topic of antibiotic resistance profiles of *Staphylococcus aureus* in Greece. A nationwide surveillance study conducted between 2012-2013 collected 1005 *Staphylococcus aureus* isolates from 30 hospitals²⁹. Main specimens' source were in order of frequency pus (57.8%), blood (31.3%) and the respiratory system (7.3%). Methicillin resistance rate was 39.1%. Molecular typing and PVL status were not examined. Concerning specifically the 393 MRSA isolates, there was only 1 Vancomycin Intermediate Resistance (VISA) isolate; however MICs for Vancomycin were >1mg/L in 57.8% and >2mg/L in 8.6% of isolates. Susceptibility to erythromycin, fusidic acid, clindamycin and moxifloxacin were 35.1%, 40.2%, 50.1% and 49.6% respectively. In contrast, susceptibility to Rifampicin and Co-trimoxazole was 93.8% and 98.9% respectively underlining the clinical value of these older generation antibiotics. Sensitivity to tetracycline and its derivatives doxycycline and minocycline was not tested. Interestingly, ceftaroline susceptibility was 82.1% (while 100% for MSSA). Susceptibility to the newer antistaphylococcal agents, linezolid, tigecycline and daptomycin was universal.

A six-year retrospective review study in the country's reference dermatological hospital examined antibiotic susceptibility of *Staphylococcus aureus* isolates from skin infections³⁰. Among 2069 isolates, MRSA represented 21%. Resistance rates for Erythromycin, Fusidic acid, and Clindamycin were 60.2%, 45.7% and 38.9% respectively. Quinolone susceptibility was not examined. Resistance rate for Rifampicin was 2.5% while

for Co-trimoxazole 12.5%. Although resistance to Tetracycline was 35.5%, it was as low as 1.6% for Minocycline. Equally low resistance was found for Gentamycin (0.7%). No resistant isolates to Vancomycin were found. Newer agents, namely Linezolid, Tigecycline, Daptomycin and Ceftaroline, were not studied.

Considering the above data, empiric treatment of community acquired pneumonia even when including a respiratory quinolone may prove inadequate in cases of MRSA etiology; moreover, clindamycin, an excellent antistaphylococcal drug, has also significant rates of resistance in Greece.

4. Conclusions

CA-MRSA pneumonia represents an uncommon but severe infection in otherwise healthy young subjects. A high level of suspicion and prompt initiation of appropriate antibiotic treatment and ICU support are crucial for a favorable outcome. Agents with both antibiotic and antitoxin effect such as linezolid, clindamycin and rifampicin are preferred. Greece has one of the highest rates of methicillin resistance among community acquired *Staphylococcus aureus* isolates. The predominant CA-MRSA clone in Greece is the SCCmec IV ST-80, also prevalent in other European countries. Practically all ST-80 isolates produce the highly pathogenic Panton-Valentine leukocidin. Spread of the ST-80 strain in the community and the hospital environment is characteristic of the continuously evolving epidemiology of *Staphylococcus aureus*. Moreover, CA-MRSA in Greece demonstrates significant rates of resistance to macrolides, fusidic acid, tetracycline, clindamycin and quinolones. In contrast resistance is low for cotrimoxazole and rifampicin and practically nil for vancomycin,

linezolid and daptomycin. Excessive antibiotic consumption both in and out of hospital is responsible. Meanwhile Greece is a country with intense population mobility due to tourism and immigration, while infrastructure and resources for infection control are inadequate. This creates a favorable milieu for development of resistant strains and eventual export to other

countries. Since *Staphylococcus aureus* has demonstrated its ability to develop antibiotic resistance and cross the barriers between hospital and community acquired infections, an active surveillance of the recorded cases and microbiological characteristics should remain a healthcare priority.

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