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ORIGINAL ARTICLE

Community-associated *Staphylococcus aureus* pneumonia among Greek children: epidemiology, molecular characteristics, treatment, and outcome

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Abstract *Staphylococcus aureus* is an infrequent cause of community-associated (CA-SA) pneumonia in children. The aim of this study was to evaluate the clinical, epidemiological, microbiological, and molecular characteristics of CA-SA pneumonia among children hospitalized in two large tertiary care referral centers during an 8-year period. Cases of CA-SA pneumonia admitted between 2007 and 2014 were retrospectively examined through medical record review. Molecular investigation was performed for available strains; *mecA*, Panton–Valentine leukocidin (PVL) (*lukS-lukF*-PV), and fibronectin binding protein A (*fnbA*) genes were detected by polymerase chain reaction (PCR). Clones were assigned by *agr* groups, pulsed-field gel electrophoresis (PFGE), SCC*mec*, and multilocus sequencing typing (MLST). In total, 41 cases were recorded (boys, 61 %), with a median age of 4.3 months (range, 1–175). Methicillin-resistant *S. aureus* (MRSA) accounted for 31 cases (75.6 %). Complications included empyema (25/41, 61 %), pneumatoceles (7/41, 17 %), and lung abscess (1/41, 2.5 %). Intensive care unit (ICU) admission was required in 58.5 %. Two deaths occurred (4.9 %). Definitive therapy was based on vancomycin with or without other antibiotics (55.9 %), followed by clindamycin and linezolid (26.5 % each). All isolates were susceptible to vancomycin (MIC_{90} 2 mg/L, range 1–2), teicoplanin, and linezolid, whereas 26.8 % were resistant to clindamycin. Among the 25 studied strains, 20 were *mecA*-positive (MRSA), carrying also the *fnbA* gene. Of these, 90 % belonged to the ST80-IV/*agr*3/PVL-positive clone. Methicillin-susceptible *S. aureus* (MSSA) strains showed polyclonality, 3/5 were PVL-positive, and 3/5 were *fnbA*-positive. MRSA and particularly the ST80-IV clone predominated among staphylococcal pneumonia cases in children. Treatment provided was effective in all but two patients, despite the relatively high minimum inhibitory concentration (MIC) of vancomycin and a high resistance to clindamycin.

Introduction

Staphylococcus aureus is a major pathogen responsible for a wide spectrum of infections, pneumonia being one of the most serious and life-threatening. It is estimated to cause 1–10 % of community-associated pneumonia (CAP) and 20–50 % of nosocomial pneumonia [1]. Community-associated methicillin-resistant *S. aureus* (CA-MRSA) has become a well-established pathogen during the last two decades, in both outpatient and healthcare settings. Community-associated *S. aureus* (CA-SA) pneumonia remains a fairly rare but serious condition both in children and adults. Panton–Valentine leukocidin (PVL) is a well-known virulence factor, implicated in staphylococcal pneumonia [2, 3]. The initial adherence of *S. aureus* to the respiratory epithelium is mediated through a group of surface adhesins called MSCRAMMS (microbial surface components recognizing adhesive matrix molecules).

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Among them, fibronectin binding protein A (FnBP) encoded by the *fnbA* gene plays an important role [4, 5]. In the USA, there has been an increase in staphylococcal pneumonia cases over the past decade among previously healthy children, particularly presenting as necrotizing pneumonia with empyema [6, 7]. The MRSA ST8-USA300 clone is predominant among these cases. Meanwhile, MRSA strains in Europe have evolved far more heterogeneously and genetically diverse, with the so-called European ST80 clone being predominant [8, 9]. Little is known about the current epidemiology of pediatric staphylococcal pneumonia in Europe, especially in Greece, with a burden of MRSA-associated invasive disease estimated at 40 % of all cases attributed to *S. aureus* [10].

We retrospectively investigated the clinical and microbiologic characteristics of CA-SA pneumonia among children hospitalized in the two large tertiary care referral centers in Athens over an 8-year period.

Materials and methods

Study design and population

The "P. & A. Aglaia Kyriakou" Children's Hospital (Institution 1) and the "Aghia Sophia" Children's Hospital (Institution 2) in Athens are two tertiary care referral centers accommodating 400 and 700 beds, respectively. Institution 1 receives 25,000–27,000 admissions, whereas Institution 2 receives 51,000–53,000 admission per year. They both serve the population of the greater Athens area comprising about 4.25 million people (2014 census), as well as other parts of central Greece. Children hospitalized with the diagnosis of primary CA-SA pneumonia in both hospitals from January 1st, 2007 to December 31st, 2014 were included in this retrospective study. Cases were identified from the microbiology laboratory database in each hospital. The demographic, clinical, and radiologic characteristics, as well as laboratory and microbiological findings, were recorded after review of medical records. Moreover, empiric and definitive treatment, complications, surgical interventions, and outcome were also recorded for each case.

Definitions

Staphylococcus aureus pneumonia was defined by isolation of the organism from pleural fluid, blood, or lung tissue from children with clinical and radiologic findings of pneumonia, with or without parapneumonic effusion. Cases with clinical signs and radiographic findings of pneumonia in which Gram stain of bronchial aspirates showed Gram-positive cocci and the organism was grown in pure culture were also included. A community-associated infection was defined according to the Centers for Disease Control and Prevention (CDC) criteria [11]. Pleural empyema was defined as pleural effusion with macroscopic presence of pus or a positive Gram stain, or culture demonstrating the presence of bacteria in the pleural space [12]. Lung abscess was considered when there was a cavitary lung lesion containing an air–fluid level on radiograph or computed tomography (CT) [13]. Viral co-infection was the detection of a viral pathogen from respiratory secretions (nasal swab/wash or tracheal aspirate).

Microbiological methods

Bacterial cultures were performed according to standard microbiological methods. Blood and pleural fluid cultures were carried out using BD BACTEC Peds Plus/F® vials in the BACTEC system (Becton, Dickinson and Company, Sparks, MD, USA). *Staphylococcus aureus* was identified by Gram stain, catalase, and coagulase production (Pastorex™ Staph-Plus, Bio-Rad, Marnes-la-Coquette, France) and the VITEK 2 Advanced Expert System (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility testing to cefoxitin, gentamicin, rifampicin, kanamycin, erythromycin, clindamycin, tetracycline, ciprofloxacin, and sulfamethoxazole–trimethoprim was performed by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, whereas for fusidic acid, the European Committee for Antimicrobial Susceptibility Testing (EUCAST) breakpoints were used. The minimum inhibitory concentrations (MICs) of vancomycin, teicoplanin, and linezolid were determined with a gradient method (Etest, bioMérieux, Marcy l'Etoile, France). MRSA were defined as those cases with cefoxitin-resistant isolates by the disk diffusion method.

Investigation for viral pathogens from nasal swabs/washes or tracheal aspirates was carried out upon admission in 12 patients in whom epidemiological and clinical data indicated a possible viral infection. Samples were examined by reverse transcription polymerase chain reaction (RT-PCR) for influenza A and B and cytomegalovirus (CMV) viruses (Cobas AmpliPrep/Cobas TaqMan CMV Test, Roche Diagnostics, Penzberg, Germany) and by direct immunofluorescence for respiratory syncytial virus (RSV), parainfluenza virus types (PIV) 1, 2 and 3 (PathoDx™ Respiratory Virus Panel, Remel Europe Ltd., Dartford, UK).

Molecular methods

Molecular investigation was performed for 25 available *S. aureus* strains, as follows.

Toxin genes and adhesion factors detection

Genes encoding PVL (*lukS-lukF*-PV) and *fnbA* were investigated by PCR [14]. PCR products were analyzed by

electrophoresis onto agarose 1 % *w/v* gels. *Staphylococcus aureus* strains ATCC49775 (PVL-positive, *agr*3), Fri 913 (*agr*1), Fri 137 (*agr*2), and HT 20000195 (*agr*4) were used as positive controls for the PCR assays [14].

Molecular typing

DNA extraction was performed into agarose disks, whereas pulsed-field gel electrophoresis (PFGE) of *Sma*I DNA digests in a CHEF-DR III apparatus (Bio-Rad Laboratories, Richmond, CA, USA) was as previously described [15]. Visual interpretation of the PFGE banding patterns and the assignments of types (pulsotypes) were performed according to the criteria of Tenover et al. PFGE types were named using capital letters. *agr* groups and SCC*mec* types were defined by PCR [14, 16]. Multilocus sequencing typing (MLST) was performed for 20 selected representative isolates according to *agr* groups, PFGE types, SCC*mec* types, gene profiles, and antibiotic resistance patterns (http://www.mlst.net). Clones were defined according to ST-SCC*mec* types.

Measurement of serum vancomycin levels

Trough levels of vancomycin in patients' serum were measured with fluorescence polarization immunoassay (Abbott AxSYM, Abbott Laboratories, Irving, TX, USA).

Statistics

Counts and percentages were reported for categorical variables, and median and interquartile range (IQR: 25th–75th percentile) for continuous variables with non-symmetric distribution, unless otherwise noted. Statistical significance of observed associations was evaluated using the χ^2 or Fisher's exact test for categorical variables and Mann–Whitney U-test or Kruskal–Wallis test for continuous variables, as appropriate. Time trends were examined using the Mann–Kendall test. All tests were two-tailed and $\alpha = 0.05$ was considered as the level of significance. Statistical analysis was performed using SPSS for Windows, version 18 (SPSS, Chicago, IL, USA).

Results

In total, 132 cases of documented bacterial CAP were recorded during the study period after review of the microbiology laboratory records. Among them, 90 (68.94 %) were due to *Streptococcus pneumoniae* and 41 (31.06 %) to *S. aureus*; MRSA accounted for 31 of the latter (75.6 %). There was an increasing trend in the number of admitted CA-SA cases per year, but the difference was not statistically significant ($p = 0.09$).

Demographics

The demographic characteristics of the patients are shown in Table 1. The median age of the cohort was 4.3 months (range, 1–175 months). Four children (9.75 %) had an underlying condition and for two of them, both younger than 2 months of age, methicillin-susceptible *S. aureus* (MSSA) pneumonia was the first manifestation of cystic fibrosis and severe combined immunodeficiency (SCID), respectively. A 19-month-old child had congenital heart disease, whereas another patient with MRSA infection had a history of central nervous system (CNS) glioma while being off chemotherapy. There was no difference in the demographic characteristics among patients infected with MRSA or MSSA strains; however, those with underlying illness were more commonly affected by MSSA ($p = 0.012$). No other difference was noted between MRSA and MSSA cases with regard to their demographic characteristics.

Radiologic, laboratory, and clinical characteristics

The radiologic and laboratory findings of patients admitted with staphylococcal pneumonia are shown in Table 2. A chest X-ray was obtained in all patients and a chest ultrasound in 24/41 (58.5 %) of cases. In six (14.6 %) children, a chest CT scan was performed. No significant difference was evident with regard to radiologic findings in relation to methicillin resistance, although all seven cases with pneumatoceles were caused by MRSA. Similarly, there was no significant difference between the two groups when white blood cell (WBC) counts were compared. However, it is remarkable that, from all six patients with leukopenia, MRSA was recovered. Patients with MRSA infection had significantly higher C-reactive protein (CRP) levels (222 vs. 110 mg/L, normal values <5 mg/L, $p = 0.012$).

Two patients (4 months and 10 years of age, respectively) with MRSA infection had pulmonary involvement during hospitalization for osteomyelitis. The first had osteomyelitis of the tibia and developed bacteremia, deep venous thrombosis (DVT), and pulmonary septic emboli with bilateral pleural fluid, while the second had acute femoral osteomyelitis and bilateral consolidation. Viral co-infections were identified in five (H1N1, PIV1, PIV3, CMV, and RSV) out of 12 tested children; however, investigation for viral infection was not systematically performed in all patients.

Microbiological and molecular analyses

Staphylococcus aureus was isolated from pleural fluid in 29 cases (70.7 %), blood in eight (19.5 %), bronchial aspirate in two (4.9 %), and from both pleural fluid and blood in another two patients (4.9 %). The percentage of concurrent bacteremia

Table 1 Demographic characteristics of patients with community-associated *Staphylococcus aureus* pneumonia

Variable	Number of patients (%)			
	All cases, $n=41$	MSSA cases, $n=10$	MRSA cases, $n=31$	*p*-Value
Gender (male)	25 (60.9)	6 (60.0)	19 (61.3)	0.94
Age (months)[a]	4.3 (2.9–28)	6 (1.7–20)	4.3 (2.9–42)	0.46
Race				
Greek	33 (80.5)	10 (100)	23 (74.2)	0.22
Roma	3 (7.3)	–	3 (9.7)	
Other	5 (12.2)	–	5 (16.1)	
Underlying disease	4 (11.4)	3 (30.0)	1 (3.2)	0.012
Hospitalization or surgery during the last year	7 (17.1)	3 (30.0)	4 (12.9)	0.211

[a] Median (25th–75th percentile)

was similar between the MRSA and MSSA groups (25.8 vs. 30 %) ($p=0.68$).

Among all isolates, MRSA (cefoxitin-resistant) accounted for 31 cases (75.6 %). All MRSA strains were resistant to fusidic acid, tetracycline, and kanamycin. None was resistant to gentamicin, rifampicin, sulfamethoxazole–trimethoprim, and ciprofloxacin. Regarding MSSA isolates, 90 % were resistant to penicillin and 20 % to sulfamethoxazole–trimethoprim. Overall, 13 (31.7 %) strains were resistant to erythromycin, 40 % among MSSA and 29 % in MRSA ($p=0.51$). Interestingly, the overall rate of resistance to clindamycin was high (11/41, 26.8 %), higher among MRSA (9/31, 29 %) as compared to MSSA (2/10, 20 %, $p=0.57$). Among them, 9/11(81.8 %) showed inducible resistance to clindamycin. The MIC_{50} of vancomycin was 1.5 mg/L, teicoplanin 1 mg/L, and linezolid 0.75 mg/L. The MIC_{90} of vancomycin was 2 mg/L (range, 1–2 mg/L), teicoplanin 1.5 mg/L (range, 0.5–2), and linezolid 1 mg/L (range, 0.25–2 mg/L). All strains were susceptible to linezolid.

Twenty-five strains were available for molecular analysis (five MSSA and 20 MRSA). All MRSA strains were *mecA*-positive, none carried *mecC*. Eighteen MRSA strains were PVL-positive and belonged to the ST80-IV/*agr*3 clone. Moreover, one PVL-positive strain belonged to ST80-IV/*agr*1 and one PVL-negative strain belonged to ST30-IV/*agr*1 clones. All were positive for *fnbA*. MSSA strains showed polyclonality. Three out of five MSSA were PVL-positive and another three positive for *fnbA* (Table 3).

Staphylococcus aureus nasal colonization was investigated in 30 (73.2 %) children and, among them, 18 (60 %) were found positive; in each patient, the colonizing strain had a phenotypically identical antimicrobial susceptibility pattern with the strain causing pneumonia.

Table 2 Radiographic and laboratory findings of patients with community-associated *Staphylococcus aureus* pneumonia

	All patients, 41 (100 %) Number of patients (%)	MSSA, 10 (24.4 %)	MRSA, 31 (75.6 %)	*P*-Value
Radiographic findings				
Consolidation	39 (95.1)	9 (90.0)	30 (96.8)	0.384
Bilateral consolidation	4 (9.8)	2 (20.0)	2 (6.5)	0.217
Empyema	25 (61.0)	5 (50.0)	20 (64.5)	0.413
Lung abscess	1 (2.4)	1 (10.0)	0	0.07
Necrotic pneumonia	7 (17.0)	0	7 (22.6)	0.09
Septic emboli	1 (2.4)	0	1 (3.2)	0.567
	Median (IQR)			
Laboratory findings				
White blood cell counts /μL[a]	16,745 (6780–25,100)	15,410 (9380–21,940)	20,200 (6260–25,400)	0.949
White blood cell counts <3000/μL[a]	6 (17.6)	0	6 (22.2)	0.220
C-reactive protein (mg/L)	199 (110–273)	110 (83–159)	222 (145–293)	**0.012**

[a] Data available for 34 patients: 7 (20.6 %) with MSSA and 27 (79.4 %) with MRSA

Table 3 Molecular investigation of 25 *Staphylococcus aureus* strains causing community-associated pneumonia

	Number of investigated strains	*mecA*	*agr*	PFGE[a] pattern	Clone (ST-SCC*mec*)[b]	*lukS-lukF*-PV	*fnbA*
MSSA	5	0	*agr*3: 3	X: 1	–	0	0
				W: 1		1	1
				V: 1		1	1
			agr NT[c]: 2	Y: 2		1	1
MRSA	20	20	*agr*3: 18	C: 18	ST80-IV: 18	18	18
			*agr*1: 1	C: 1	ST80-IV: 1	1	1
			*agr*1: 1	A: 1	ST30-IV: 1	0	1

[a] Pulsed-field gel electrophoresis

[b] Sequence type-staphylococcal cassette chromosome

[c] *NT* not typeable

Management and outcome

Data regarding antimicrobial treatment and type of management are shown in Table 4.

Empirical therapy consisted of beta-lactam with or without other antibiotics for 31 among 37 recorded cases (83.8 %), whereas clindamycin was used alone or as part of therapy in 15 (40.5 %) cases. Vancomycin was given as part of therapy in 17 (45.9 %) cases.

Definitive therapy solely with vancomycin was administered to eight (21.6 %) patients, all with MRSA pneumonia, for 15–25 days. The vancomycin MIC was 2 mg/L for four of these cases; nevertheless, the final outcome was favorable. Clindamycin alone or in combination with other antibiotics was used in ten children (27 %) and linezolid in 12 (32.4 %), among which ten with MRSA pneumonia. For MSSA cases, antistaphylococcal penicillin was used as standard of care in seven children and linezolid was used for two children who were admitted in the pediatric intensive care unit (PICU). Trough levels for vancomycin were measured only in four (25 %) of the vancomycin-treated cases and they were below 15 μg/L in two and between 15–20 μg/L in the other two cases.

Two deaths occurred, both due to MRSA. A 5-month-old boy with a preceding influenza-like illness who was positive for PIV1 developed necrotizing pneumonia and acute respiratory distress syndrome requiring mechanical ventilation and died on the third hospitalization day in the ICU. The second patient was a 14-year-old girl with post-H1N1 staphylococcal pneumonia. Both had leukopenia on admission (WBC counts of 900/μL and 1000/μL, respectively). Patients with MSSA infection had a significantly longer duration of hospitalization (26.5 vs. 21 days, $p = 0.04$). However, this difference did not reach significance when the two patients who deceased soon after their admission (on the first and third days of hospitalization, respectively) were excluded from the analysis (26.5 vs. 22 days, $p = 0.06$).

Table 4 Clinical course and management strategies of community-associated *Staphylococcus aureus* pneumonia cases by methicillin status

	All patients 41 (100) Number of patients (%)	MSSA 10 (24.4)	MRSA 31 (75.6)	*p*-Value
Management and outcome				
Chest tube placement	32 (78)	7 (70)	25 (80.6)	0.40
Open thoracotomy	5 (12.2)	2 (20)	3 (9.7)	0.39
Video-assisted thoracoscopic surgery (VATS)	1 (2.4)	1 (10)	0	
Length of stay in the hospital (days)	23 (17–27)	26.5 (23.5–32.0)	21 (15–25)	**0.04**
PICU admission	24 (58.5)	6 (60.0)	18 (58.1)	0.91
Death	2 (4.87)	0 (0)	2 (6.45)	0.41
Definitive antimicrobial treatment[a]				
Antistaphylococcal beta-lactam	7 (18.9)	7 (77.8)	0 (0)	**<0.0001**
Vancomycin treatment with or without other antibiotics	16 (43.2)	1 (11.1)	15 (53.6)	**0.025**
Vancomycin-only treatment	8 (21.6)	0 (0)	8 (28.6)	0.07
Clindamycin treatment with or without other antibiotics	10 (27)	2 (22.2)	8 (28.6)	0.70
Clindamycin-only treatment	3 (8.1)	0 (0.0)	3 (10.7)	0.30
Linezolid treatment	12 (32.4)	2 (22.2)	10 (35.7)	0.45

[a] Data available for 37 patients; missing data for one MSSA and three MRSA pneumonia cases

There was no statistically significant difference on disease severity, procedural intervention, and the outcome of pneumonia between patients with PVL-negative and PVL-positive strains, whereas children with underlying disease were prone to having pneumonia caused by a PVL-negative strain ($p = 0.012$).

Discussion

In pediatric CAP, *S. pneumoniae* and *S. aureus* were the most commonly isolated bacterial pathogens for our population; this is in accordance with a similar study from India where *S. aureus* was found to be an important pathogen [17]. Our study focused on the investigation of factors predisposing to *S. aureus* pneumonia in children and correlated them to virulence factors of the bacterium. We retrospectively reviewed a considerable cohort of community-associated *S. aureus* pneumonia cases hospitalized in the two largest pediatric hospitals in Greece, a country with a heavy burden of MRSA-associated disease [10].

To the best of our knowledge, this is a unique series of pediatric pneumonia cases in Europe. Although there are a few reports concerning pediatric community-acquired *S. aureus* pneumonia from the USA, relevant data from Europe are scarce and only a few case reports have been published [18–20].

Most of our patients were boys and young infants, with a median age of 4.3 months, in accordance with the first documentation from Chartrand and McCracken in 1982, who reported a median age of 6 months among patients with staphylococcal pneumonia [21]. Thus, *S. aureus* pneumonia remains predominantly a disease of young infants. No significant difference according to age and race was found regarding the status of methicillin resistance. Similar results were observed regarding distinct radiologic entities and complications, including multilobular infiltrates, pneumatoceles, empyema, abscess, and evolution to necrotizing pneumonia.

A predominance of MRSA was noted as the cause of staphylococcal pneumonia in our cohort, in which most children had no underlying illness. This is in accordance with recent data from the USA [6], where the prevalence of MRSA was 74 % among 117 staphylococcal pneumonia cases. According to recent data from Institution 1, the rate of MRSA strains involved in pneumonia was higher as compared to other types of invasive staphylococcal disease (72 vs. 44.4 %).

Staphylococcus aureus can invade the lung parenchyma directly through the tracheobronchial tree or through the hematogenous route. In this report, there were two cases of pulmonary involvement concurrently with osteoarticular infection, both due to MRSA. No evidence of endocarditis was found in these patients. Pulmonary involvement in children with staphylococcal osteomyelitis is not uncommon, especially in MRSA-associated disease [7].

From previously published data covering a 15-year period, it is evident that the European ST80-*mecA*-PVL-positive clone is predominant in Greece and accounts for the vast majority of MRSA infections [9, 22]. PVL leukocidin forms pores in the cell membrane, causing lysis of the host's neutrophils, monocytes, and macrophages, inducing a significant release of inflammation mediators. This clone is characterized by resistance to fusidic acid, kanamycin, and tetracycline [9, 23], and was predominant in the present cohort of pediatric pneumonia cases among MRSA strains. It is generally accepted that PVL-positive *S. aureus* strains exhibit a tropism for the lower respiratory tract [3]. However, it seems that PVL is a virulence factor for severe staphylococcal pneumonia, regardless of methicillin resistance. We did not observe any significant difference in the duration of hospital stay, rate of admission to the PICU, and requirement for surgical intervention in association with methicillin resistance. Remarkably, all MRSA isolates except for one and 3/5 MSSA strains causing pneumonia in the present study were PVL-positive. Thomas et al. reported a case series where community-acquired PVL-producing MSSA strains caused pneumonia with complications, such as pneumatoceles, recurrent bilateral multilobar infiltrates, pneumothoraces, empyema, lung abscess, and diaphragmatic paralysis [24]. Diep et al., in 2010, found a striking epidemiological association of PVL with serious staphylococcal infections, using a rabbit model of necrotizing pneumonia [25].

All but two of the examined staphylococcal strains in this study were positive for the *fnbA* gene encoding fibronectin binding proteins (FnBPs). Microbial adherence to cells and matrix components promotes colonization and infection. Mongodin et al. have shown that *S. aureus* prefers mainly undifferentiated human airway epithelial cells (HAECs) for adhesion through FnBPs, whereas protein A (SpA) and clumping factor (ClfA) do not play a role in this process. Fibronectin acts as a bridging molecule between cellular a_5b_1-integrins and staphylococcal FnBPs, inducing, thus, the adherence of the microbe to epithelial cells, fibroblasts, and subsequent internalization of the pathogen [4]. In the same study, among 32 *S. aureus* strains from cystic fibrosis lungs and nosocomial pneumonia, 97 % possessed the *fnbA* and *fnbB* genes.

Necrotizing pneumonia is an uncommon complication of CAP in children. Although it can also be caused by *S. pneumoniae*, it is most commonly associated with PVL-positive staphylococcal infection. In a recent study from France, a bacterial agent was identified in 21/41 patients of necrotizing pneumonia and *S. aureus* accounted for 13/21 cases. Twelve of these 13 cases were caused by MSSA PVL-positive strains and one by MRSA [20].

The mortality rate in the present study was low (4.9 %) as compared to 39 %, a rate reported in a series of 133 community-associated PVL-positive staphylococcal pneumonia cases from France between 1986 and 2010, which included children and adults with a median age of 22 years. In the same study, mortality was not related to methicillin resistance but to the presence of erythroderma, airway bleeding, and leukopenia [26]. In a recent study from Texas, USA that included 119 children with staphylococcal pneumonia, the mortality was 0.85 % [6]. In a series of 14 PICU-admitted children with MRSA pneumonia or pulmonary involvement from Tunisia over a 9-year period, the observed mortality was 14.3 % [27]. Lethal cases of necrotizing MRSA pneumonia have been linked to antecedent viral respiratory infection, with influenza being the prototype infection [28, 29]. Influenza infection was documented in one of the two lethal cases in our series.

Of note, it has been recently suggested that low leukocyte count is a marker of poor prognosis, allowing early identification of severe cases and appropriate management. In a French case series of 148 cases of childhood and adulthood necrotizing pneumonia due to PVL-positive *S. aureus*, the mortality rate was 75.8 % for the 62 cases with leukocyte count below 3000/μL [30]. The same is suggested by our findings as mortality was high among patients with leukopenia (2/6, 33.3 %), and both patients who died had leukopenia.

The majority of our cases required an invasive procedure to drain the pleural exudate or, in addition, decortication to excise the fibrinous pleural peel for the resolution of complicated pneumonia. Conservative management only with antibiotics is not adequate and prolongs hospitalization [31].

Our study shows a high resistance rate of pneumonia-causing strains to clindamycin (26.8 %) in comparison with the average resistance rate (15.6 %) of all MRSA clinical isolates ($n = 1083$) in institution 1 for the period 2007–2013 (unpublished data). Although clindamycin is recommended as an option for empiric therapy among children with possible staphylococcal pneumonia, the disproportionately high resistance rate observed indicates that this antibiotic should not be used empirically as monotherapy in these settings.

There is growing evidence that antimicrobial agents which inhibit the microbial ribosome system, such as the protein synthesis inhibitors clindamycin and linezolid, suppress the synthesis of bacterial toxins. In addition to alpha-hemolysin (Hla) and protein A (SpA), which are well-known mediators of staphylococcal disease, PVL is a toxin almost ubiquitously present in CA-MRSA strains causing pneumonia, bone, and skin and soft tissue disease. Otto et al. have recently shown that clindamycin suppresses PVL, Hla, and SpA mRNA levels, and subinhibitory concentrations of clindamycin decreased the levels of the three mRNAs studied [32]. Linezolid reduced the mRNA and protein expression of PVL and SpA, though to a lesser extent than clindamycin. Exposure to subinhibitory concentrations of vancomycin had no significant effect on PVL, Hla, and spA mRNAs. It is, therefore, suggested that clindamycin and linezolid can suppress staphylococcal virulence factors in serious infections. However, there are no clinical studies to document an improved outcome over vancomycin with the inclusion of these antibiotics in the therapeutic regimen against MRSA CAP. Furthermore, linezolid has not been endorsed as a first-line treatment and is considered as an alternative agent by the Infectious Diseases Society of America (IDSA) guidelines for the treatment of MRSA-associated CAP in infants and children older than 3 months of age [33]. The choice of linezolid over vancomycin in MRSA CAP is still inconclusive, since data from clinical trials comparing different antibiotics for this form of pneumonia are not available [34].

A limitation of our study is that it was conducted retrospectively, compromising the uniform access to all medical information. Moreover, testing for respiratory viruses was not routinely sought for every single case and the point outcome was “discharged alive” and long-term follow-up was not available.

In conclusion, the European ST80-IV-PVL-positive MRSA clone predominates among community-associated *S. aureus* pneumonia cases in our settings, although the overall staphylococcal pneumonia prevalence is not high. Clinicians should be aware that PVL-positive staphylococcal pneumonia can be lethal, even among previously healthy children. Optimal treatment includes prompt pleural drainage, together with proper antibiotic therapy. Treatment provided was effective in all but two patients, despite the relatively high MIC of vancomycin (≥1 mg/L) in all cases. The knowledge of high local resistance rate to clindamycin warrants broad coverage when treatment is empirically initiated. Appropriate modification can be applied as definitive treatment, after the antimicrobial susceptibility testing is available to the clinician.

Compliance with ethical standards

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Conflict of interest E. Drougka has received funding from the National Staphylococcal Reference Laboratory.

M. Tsolia has received investigator-initiated grants from Pfizer and GSK. She has also received honoraria for participation in Advisory Boards of GSK, Pfizer, and Novartis, and for presentations in sponsored symposia by GSK, Pfizer, and Sanofi Pasteur.

For the remaining authors, no conflicts of interest were declared.

Informed consent Informed consent was obtained from all individual participants included in the study.

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