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Infectious Spondylitis-Associated *Staphylococcus aureus* with Virulence Gene *pvl* or *tst* Causes More Necrosis than Apoptosis in Human Alveolar Basal Epithelial Cell Line A549

Tsung-Jen Huang[1,2,3,4], Chi-Han Lee[5], Meng-Huang Wu[1,3,4], Yen-Yao Li[1,2], Tsung Han Yang[6], Chin-Chang Cheng[1,2], Ching-Yu Lee[1], Chih-Cheng Lu[5], Chishih Chu[5*]

[1]Department of Orthopedic Surgery, Chang Gung Memorial Hospital, Chiayi Branch, Taiwan
[2]College of Medicine, Chang Gung University, Taiwan
[3]Department of Orthopedic Surgery, Taipei Medical University Hospital, Taiwan
[4]School of Medicine, Taipei Medical University, Taiwan
[5]Department of Microbiology, Immunology, and Biopharmaceutics, National Chiayi University, Taiwan
[6]Department of Laboratory Medicine, Chang Gung Memorial Hospital, Linkou Branch, Taiwan
Email: *cschu@mail.ncyu.edu.tw

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Abstract

Methicillin-sensitive and resistant *Staphylococcus aureus* (MSSA and MRSA, respectively) can cause non-tuberculosis infectious spondylitis. In 43 cases of bacterial infectious spondylitis, *Mycobacterium tuberculosis* and *S. aureus* were the two major causative pathogens. MRSA caused more anterior operations and thoracic infections, while MSSA caused more posterior infections and lumbar infections. Differences between six *S. aureus* isolates from infectious spondylitis were characterized. MLST and staphylococcal cassette chromosome *mec* (SCC*mec*) analysis identified MSSA ST959 and ST30 isolates, MRSA ST239/SCC*mec* IIIA isolates 2 and 3, ST59/SCC*mec* IIIA-like isolate 6, and ST30/SCC*mec* IV isolate 5. While all of the isolates were resistant to penicillin and ampicillin, the MRSA isolates were more resistant than the MSSA isolates. Carbapenem-resistant MRSA ST239/SCC*mec* IIIA and ST59/SCC*mec* IIIA-like isolates of the *agr1* type were also resistant to clindamycin and erythromycin. Leukocidin genes (*pvl* or *lukED*) and hemolysin genes (*hla*, *hld* and *hlg*) were present in all of the isolates. All six isolates caused more necrosis than apoptosis in the human alveolar basal epithelial cell line A549; however, ST59/SCC*mec* IIIA-like isolate 6, ST30/

*Corresponding author.

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SCC*mec* IV isolate 5 with *pvl* genes, and MSSA ST30 isolates with *tst* caused greater than 40% cell death after the 4-h incubation. Regardless of the MRSA isolate and its SCC*mec* type or the MSSA isolate, the infectious spondylitis-associated *S. aureus* isolates differed genetically, and the *pvl* and *tst* genes may be important genes for cell necrosis.

Keywords

Infectious Spondylitis, *Staphylococcus aureus*, Virulence Factor, MLST, Necrosis, Apoptosis

1. Introduction

Infectious spondylitis is difficult to diagnosis due to its latent symptoms and is caused by direct iatrogenic inoculation of methicillin-resistant *Staphylococcus aureus* (MRSA)-related thoracic spondylitis after cervical spine surgery [1] or distant infections of the genitourinary tract, skin and soft tissue, intravascular devices, gastrointestinal tract, respiratory tract, and oral cavity [2]. Pyogenic vertebral osteomyelitis has an overall mortality rate of up to 11% [3]. Greater than 60% of infectious spondylitis cases occur in males and are caused by bacteria [4]. The two major types of bacterial infectious spondylitis are tuberculosis, which is caused by *Mycobacterium tuberculosis* and is the most common cause of spinal infection (with a prevalence ranging from 9% - 46%), and non-tuberculosis, of which *S. aureus* is the predominant pathogen worldwide and accounts for 20% - 84% of cases [4]-[7].

In pyogenic spondylodiscitis, the main underlying diseases in an aging group have been shown to be diabetes, malignant tumors and pyelonephritis and the pathogens have included *Enterobacteriaceae* (7% - 33%), such as *Escherichia coli* in Japan [7], coagulase-negative staphylococci (CNS) (5% - 16%), such as *Staphylococcus epidermis*, and *Streptococcus* spp., such as viridian as well as group C *Streptococcus* and *S. agalactiae* [6]. Additionally, the pathogens differ between community-acquired (CA) and hospital-acquired (HA) infections. Gram-positive bacteria, including *S. aureus*, *Streptococcus intermedius*, CNS, and *S. agalactiae*, are responsible for CA infections (10/12, 83.3%), and MRSA and gram-negative bacteria are responsible for HA infections [8].

In MRSA, CA-MRSA isolates carry the staphylococcal cassette chromosome *mec* (SCC*mec*) IV element, whereas HA-MRSA isolates consist of SCC*mec* II and III [9]. Furthermore, the major virulence factors of *S. aureus* include Panton-Valentine leukocidin (PVL), γ-hemolysin (Hlg), toxic shock syndrome toxin 1 (TSST-1), and exfoliatin A (ETA) and B (ETB). In Taiwan, multi-locus sequence typing (MLST) analysis of a nasal carriage and community-onset infection determined ST188 of the clonal complex 1 (CC1) as the predominant virulent clone of the MSSA isolate and ST59 as the common ST type for MSSA and MRSA [10]. In addition, major ST types for necrotizing fasciitis-associated CA and HA *S. aureus* are ST59 and ST239 [11]. In this study, microbial associated infectious spondylitis and the characteristics and pathogenesis of six *S. aureus* isolates were investigated.

2. Materials and Methods

2.1 Study Design

This experiment was approved by the institutional review board (IRB) of the Chang Gung Memorial Hospital (CGMH, IRB 98-0675B), and informed consent was obtained from all of the patients who were hospitalized at the 1000-bed Chiayi CGMH in southern Taiwan from 2010 to 2012. In total, 43 cases of bacterial infectious spondylitis were enrolled, and the bacterial species were identified at the Department of Anatomic Pathology and Laboratory Medicine, Chang Gung Memorial Hospital, Chiayi Branch. Among these, six patients with infectious spondylitis infected by *S. aureus* were retrospectively analyzed for factors including age, gender, infection site; moreover, comorbid underlying chronic conditions, such as diabetes mellitus, hypertension, chronic liver disease, chronic renal insufficiency, chronic obstructive pulmonary disease, and malignancy, were investigated. Additionally, infectious pathogens, empiric antibiotics, the number of operative debridements and reconstructions, the duration of hospitalization, and the in-hospital mortality rate were evaluated.

2.2. Identification and Genetic Analysis of *Staphylococcus aureus* Isolates

Six infectious spondylitis-associated *S. aureus* isolates were identified in the hospital laboratory and the university. The bacteria were first analyzed by coagulase testing and Gram staining. Furthermore, *S. aureus* was identified by PCR amplification of the *S. aureus*-specific *clf*A, 16S rRNA, and *nuc* genes, as previously described [12] [13]. The genotype of each isolate was determined by *Sma*I-digested PFGE analysis, according to a previously reported method [14]. Briefly, whole-cell embedded agarose plugs were digested with the restriction endonuclease, *Sma*I (New England Biolabs, Ipswich, MA, USA). The DNA fragments were resolved by a CHEF DR-III apparatus (Bio-Rad, Hercules, CA, USA). The isolates were defined as a subgenotype for those with ≤ 3 fragment differences and as a genotype for those with more than 3 fragment differences. The MLST types for each isolate were determined according to the method described by Enright *et al*. [15] and by analysis of MLST databases (http://saureus.mlst.net).

2.3. Antimicrobial Susceptibility Testing

The susceptibility of the six *S. aureus* isolates to the following antimicrobials was examined: AMP (10 μg), CEF (30 μg), CIP (5 μg), CLI (2 μg), ertapenem (10 μg), ERY (15 μg), IPM (10 μg), MEM (10 μg), OXA (1 μg), oxytetracycline (30 μg), PEN (10 units), TET (30 μg), and SXT (1.25 μg for trimethoprim and 23.75 μg for sulfamethoxazole) (Becton Dickinson, Spark, MD, USA). Susceptibility analysis was performed using the disc diffusion method and the guidelines of Clinical and Laboratory Standards Institute (CLSI) [16]. The *S. aureus* isolates BCRC10781 and BCRC15211 were used as OX-susceptible and resistant reference strains, respectively.

2.4. PCR Identification of SCC*mec* Types and Genes for the Virulence Factors PVL, Hlg, TSST-1, ETA, and ETB

SCC*mec* types I-IV were identified by multiplex PCR amplification [17] [18]. If not groupable into types I-IV, isolates were grouped into SCC*mec* type V or VT (or VII) by the PCR detection of *ccrC* (*ccr*5) homologues [19]-[21]. Genes for virulence factors PVL, Hlg, TSST-1, ETA, and ETB were identified by simplex and multiplex PCR amplifications using primers described elsewhere [22] [23]. Accessory gene regulator (*agr*) typing was also performed, as described previously [24].

2.5. Cytotoxicity of Clinical *S. aureus* Isolates against Cell Line A549

Human alveolar basal epithelial cell line A549 was routinely cultured in RPMI-1640 medium with 10% FBS. After the transfer of 5×10^5 A549 cells into each well of a 24-well plate (Becton, Dickinson and Company), the isolate (1×10^7 cfu) was added to reach MOI = 20. The mixtures were incubated for 1 or 4 h at 37˚C and then washed twice with PBS. The cells were treated with 0.25% trypsin/0.53 mM EDTA for 5 min until cell detachment, and then 800 μl of RPMI-1640 medium with 10% FBS was added. The cells were then pelleted at 3000 rpm for 10 min. After washing with PBS, the cells were stained with propidium iodide (PI) and annexin V using an Annexin V-FITC Apoptosis Detection Kit (Cat. No. AVK250, Strong Biotech Corporation, Taiwan) for 10 - 15 min in the dark. The fluorescence of PI and annexin V in the cells was measured by flow cytometry, and the data were analyzed using WinMDI software.

3. Results

3.1. Clinical Manifestation and Bacterial Species

S. aureus-associated infectious spondylitis was only observed in male patients with an infection in the lumbar spine (five patients) and thoracic spine (one patient) With the exception of one patient without any underlying disease, diabetes mellitus and renal disease were found in four of the patients, followed by hepatitis C infection and cellulitis in three patients, and gastric ulcer, septic shock, and arterial disease in two patients. *S. aureus* infection was not correlated with the duration of hospitalization, previous amputation or mortality. With a change in the antibiotic use, patient 3, who had eight underlying diseases, died. Patients 2 and 5, who had MRSA infections, received two and four spine debridements and reconstructions, respectively.

Among 43 bacterial infectious spondylitis, *M. tuberculosis* and *S. aureus* accounted for 30.2% (13 cases) and 41.9% (19 cases), respectively (**Table 1**). The remaining species included coagulase-negative *Staphylococcus*,

Table 1. Retrospective analysis of 43 cases of bacterial infectious spondylitis.

Bacterial species	Number (%)	OP			Sex		Age		location			
		A	P	A + P	F	M	Range	Mean	L	T	L + S	L + T
M. tuberculosis	13 (30.2)	10	1	2	4	9	34 - 83	66.6	7	4	1	1
S. aureus	18 (41.9)	14	4		7	11	29 - 81	58.3	7	9	1	1
MRSA	10 (23.3)	9	1		4	6	43 - 80	59.5	3	7		
MSSA	8 (18.6)	5	3		3	5	29 - 81	56.7	4	2	1	1
Others	12 (27.9)	7	5		6	6	33 - 83	61.8	11	1		
Total	43 (100)	31	10	2	17	26	29 - 83	61.7	25	14	2	2

A: anterior; P: posterior; L: lumbar; T: thoracic; L + S: lumbar and S segment.

E. corrodens, *E. coli*, *K. pneumonia*, *Micrococcus* spp., *Peptostreptococcus* spp., *Prevotella* spp., *Proteus mirabilis and S. enterica serogroup D.* Anterior operations (72.1%) were more prevalent than posterior operations (23.3%). *M. tuberculosis* and *MRSA* caused more anterior operations, while MSSA caused more posterior operations. Infection sites differed among species that mainly infected a single site in the lumbar (58.1%) or thoracic (32.6%) spine. More lumbar infections were observed for other species (91.7%, 11/12), followed by MSSA (75%), *M. tuberculosis* (69.2%), and MRSA (30%). Infectious spondylitis occurred more in males than in females (1.5:1), with a ratio of 2.25 for *M. tuberculosis*, 1.5 for MRSA (1.5:1) and 1.67 for MSSA, but was equally distributed for other species.

3.2. Antimicrobial Resistance and SCC*mec* Type

All six *S. aureus* isolates were resistant to PEN and AMP (**Table 2**). Among the 5 MRSA isolates, SCC*mec* type IIIA isolates 2 and 3 were resistant to all 11 antimicrobials examined, while the MRSA SCC*mec* type IIIA-like isolate 6 was only resistant to 7 of the tested antimicrobials and lacked PCR products for C and F of the normal SCC*mec* IIIA type. Additionally, SCC*mec* type IV isolate 5 was less resistant than the other three isolates. While the MRSA SCC*mec* type IIIA and IIIA-like isolates were resistant to at least one of the two carbapenems (ertapenem and IMP), the SCC*mec* type IV isolate appeared to have a reduced susceptibility to ETP but was susceptible to two other carbapenems (IMP and MEM).

3.3. Genomic and Genetic Variations

MLST analysis revealed that MSSA isolates 1 and 4 belonged to ST959 and ST15, respectively, and four MRSA isolates belonged to ST239 (isolates 2 and 3), ST30 (isolate 5) and ST59 (isolate 6) (**Table 3**). In addition to being identical in ST239 and antimicrobial patterns, pulsotype analysis demonstrated that isolates 2 and 3 differed with respect to pulsotypes (**Figure 1(a)**). Plasmid analysis revealed that isolates 1 to 4 carried one plasmid of 50-kb or a less than 6.6-kb plasmid, and isolates 5 and 6 lacked a plasmid (**Figure 1(b)**). The *agr* type analysis indicated that all MRSA SCC*mec* type IIIA and IIIA-like isolates belonged to *agr* 1, and the other isolates belonged to a separate *agr* type.

3.4. Virulence Gene Analysis

Among the virulence genes examined, all of the isolates carried leukocidin genes; *lukED* was identified in isolates 1 to 4, and *pvl* was observed in isolates 5 and 6 (**Table 3**). The hemolysin genes *hla*, *hld* and *hlg* encoding *α*, *δ*, and *γ*-hemolysin, respectively, were identified in all of the isolates, while β-hemolysin was only identified in isolate 6 (**Table 3**). Within the two *hlg* types, five isolates carried *hlg*-2. The exfoliative toxin genes *eta* and *etb* were not found, while the toxic shock syndrome toxin gene, *tst*, was only observed in MRSA isolate 5.

Table 2. SCC*mec* type and antimicrobial susceptibility of *S. aureus* isolates.

Isolate	SCC*mec* type	C (II, III)	D (I, II, IV)	E (III)	F (III)	H (IIIA)	Susceptibility testing[a]											
							P	AMP	OX	CF	TE	OT	CC	E	SXT	ETP	IMP	MEM
1					+		R	R	S	S	R	R	I	I	S	S (0.125)	S (0.016)	S (0.094)
2	IIIA	+		+	+	+	R	R	R	R	R	R	R	R	R	R (>32)	R (>32)	I (12)
3	IIIA	+		+		+	R	R	R	R	R	R	R	R	R	R (>32)	R (>32)	I (12)
4							R	R	S	S	R	R	S	S	S	S (0.125)	S (0.023)	S (0.094)
5	IV		+		+		R	R	R	I	S	S	I	I	S	I (4)	S (0.064)	S (2)
6	IIIA-like			+			R	R	R	S	S	S	R	R	S	R (>32)	S (2)	S (6)

[a]P: penicillin, AMP: ampicillin, OX: oxacillin, CF: cephalothin, TE: tetracyclin, OT: oxytetracycline, CC: clindamycin E: erythromycin, SXT: trimethoprim/sulfamethoxazole, ETP: ertapenem, IMP: imipenem and MEM: meropenem. The susceptibility to ETP, IMP, and MEM was determined by Disc diffusion and minimum inhibitory concentration (MIC) methods; the number in the parenthesis is the MIC value (μg/mL).

Table 3. MLST and toxin gene analysis of *Staphylococcus aureus* isolates.

Isolate	Plasmid (kb)	Pulsotype	ST type	Haemolysis	Leukocidin genes		Hemolysin genes[a]					*tst*	*agr* type			
					pvl	*lukED*	*hla*	*hlb*	*hld*	*hlg*	*hlg*-2		*agr* 1	*agr* 2	*agr* 3	*agr* 4
1	50	III	959	*α*		+	+		+		+					+
2	<6.6	II	239	*γ*		+	+		+		+		+			
3	<6.6	IIA	239	*γ*		+	+		+		+		+			
4	50	IIIA	15	*γ*		+	+		+		+			+		
5	None	I	30	*α*	+		+		+	+		+			+	
6	None	IV	59	β	+		+	+	+		+		+			

[a]*hla*, *hlb*, *hld*, *and hlg* (*or hlg*-2): hemolysin genes *α*, β, *δ* and *γ*, respectively; *tst*: toxic shock syndrome toxin 1; *agr*: accessory gene regulator. *eta* and *etb* was not found in any isolate.

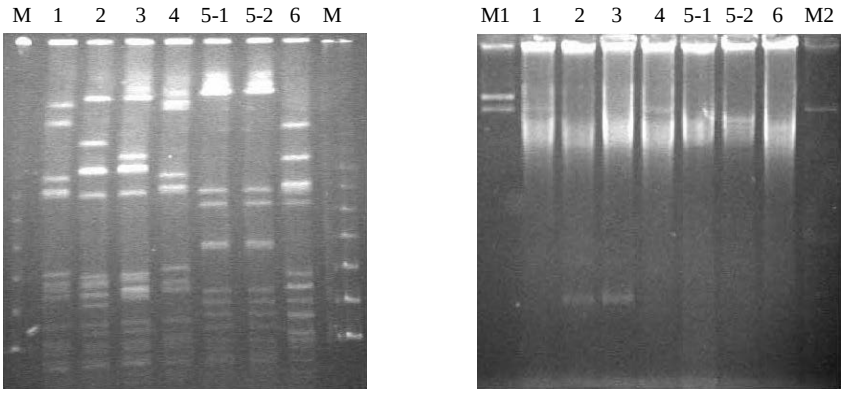

Figure 1. Genetic analysis of six spondylitis-associated *S. aureus* isolates. (a) Pulsed-field gel electrophoresis (PFGE) of *sma*I-digested macro-fragments of the six isolates 1 - 6. M: *λ* chromosomal DNA marker. (b) Plasmid analysis of six isolates 1 - 6. M1: *S. choleraesuis* OU7526, which carries the 50-kb and 90-kb plasmids. M2: *S. choleraesuis* OU7085, which carries the 6-kb and 50-kb plasmids.

3.5. Clinical *S. aureus* Associated the Cell Death Types of Cell Line A549

Infectious spondylitis can result from distant infections, such as respiratory tract. Therefore, we used the human alveolar basal epithelial cell line A549 to investigate the cytotoxicity of *S. aureus* to epithelial cells. Although six *S. aureus* isolates resulted in different cell death rates of cell line A549 by necrosis, early and late apoptosis, all clinical isolates caused cell death, with over a 40% cell death rate for isolates 4, 5, and 6 at the 4-h incubation point, whereas the rate of cell death did not differ among the bacteria at the 1-h incubation point (**Figure 2**). While isolates 2 and 3 resulted in the lowest cell death rates (similar to that caused by *E. coli* pir116), the remaining four isolates showed increased necrosis compared with the control *S. aureus* ATCC25923. Analysis of the cell death types between 1 hour and 4 hours demonstrated that isolates 4 - 6 caused more cell death than did other isolates, and all six clinical isolates increased necrosis more than late apoptosis, with a ratio larger than 1.27 compared with *E. coli* pir116 (0.53) and *S. aureus* ATCC 25923 (0.34) (**Table 4**).

4. Discussion

The prevalence of spinal infection varies and is dependent on the bacterial species, patients and investigators. Spondylodiscitis is observed at equal rates between CA and HA infections and is frequently associated with lumbosacral infection (72.8%), followed by thoracic and cervical tract infections [25]. The major pathogens are *S. aureus* (28%, 43.1%; 8 MRSA cases), coagulase-negative staphylococci (CNS) (8%, 12.3%), and *Pseudomonas aeruginosa* (8%, 12.3%). Another study of spinal infection cases has shown them to be caused by *S. aureus* (43.1% MRSA cases), gram-negative cocci, *Mycobacterium tuberculosis*, coagulase-negative *Staphylococcus* (12.3%), and *P. aeruginosa* (12.3%) [26]. In bacteremic vertebral osteomyelitis, *S. aureus* (82%) is the predominant pathogen for CA infections [27]. In the present study, *S. aureus* and *M. tuberculosis* were the major pathogens to cause infectious spondylitis and caused more infections in males; furthermore, *M. tuberculosis* and MSSA preferred to infect lumbar sites, whereas MRSA caused more thoracic infections (**Table 1**). Apparently, MRSA and MSSA differ in causing spinal infections.

In a study of patients with mycotic aneurysm and/or infectious spondylitis, *Salmonella* (18%, 34,6%) and *Klebsiella pneumoniae* (6%, 11.6%) of gram-negative bacteria (28%, 53.8%) and *S. aureus* (6%, 11.6%) and Viridans streptococcus (5%, 9.6%) of gram-positive bacteria were the major pathogens in 52 cases of mycotic

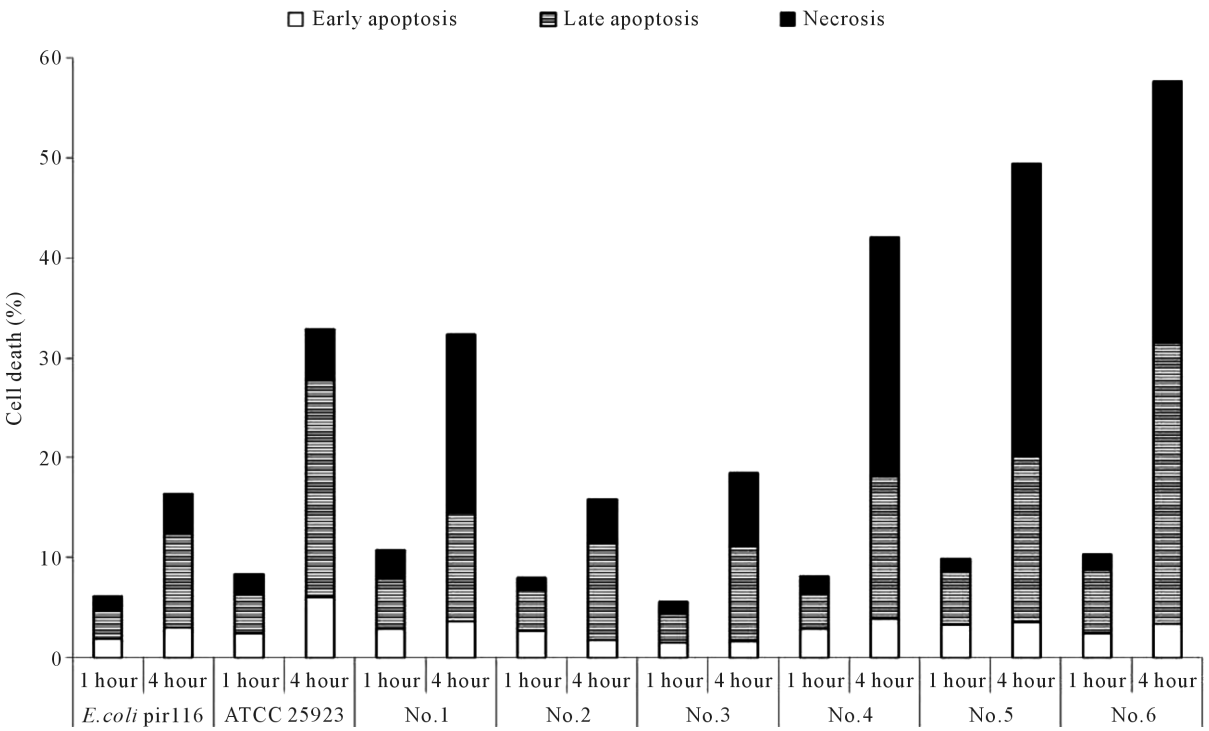

Figure 2. The percentage of A459 cell death types caused by *S. aureus* isolates at two periods.

Table 4. The ratio of A459 cell death types caused by *S. aureus* isolates between two periods.

Strain	Apoptosis (%)			Necrosis	Total cell death	Ratio of necrosis over total apoptosis
	Early	Late	Total			
E. coli pir116	1.59 (32.3%)	3.33 (67.7%)	4.92	2.75	7.67	0.53
S. aureus ATCC 25923	2.46 (30.9%)	5.51 (69.1%)	7.97	2.69	10.66	0.34
No. 1	1.28 (38.0%)	2.09 (62.0%)	3.37	6.39	9.76	1.90
No. 2	0.66 (22.0%)	2.33 (78.0%)	2.99	3.80	6.79	1.27
No. 3	1.13 (26.3%)	3.17 (73.7%)	4.30	6.76	11.06	1.57
No. 4	1.38 (30.0%)	2.88 (70.0%)	4.26	14.65	18.91	3.44
No. 5	1.08 (25.9%)	3.08 (74.1%)	4.16	23.80	27.96	5.72
No. 6	1.40 (24.0%)	4.44 (76.0%)	5.84	16.55	22.39	2.84

aneurysm without infective spondylitis, in contrast with four *Salmonella* infections, one *Streptococcus pyogenes* infection and one *S. aureus* infection in six cases of spontaneous infectious spondylitis and mycotic aneurysm [10]. *S. aureus* infection significantly increased infectious complications, such as psoas, paravertebral abscesses and epidural abscesses in patients with vertebral osteomyelitis [28]. Additionally, MRSA caused more frequent persistent bacteremia and relapse compared with MSSA in hematogenous vertebral osteomyelitis [29]. Our MSSA isolates were ST959 and ST15, which differed from the major virulent clone ST188 of CC1 of MSSA [10]. In MRSA, the ST239 and ST59/SCC*mec* IIIA isolates and ST30/SCC*mec* IV isolates are responsible for infectious spondylitis (**Table 2** and **Table 3**) and necrotizing fasciitis-associated *S. aureus* infection [11].

Isolation of markers and identification methods for infectious spondylitis may provide useful information for treatment. Based on an analysis of percutaneous endoscopic discectomy and drainage (PEDD) and computed tomography (**CT**)-guided biopsies, PEDD identified more causal pathogens than CT-guided biopsy [90% (18/20) vs. 47% (15/32)] [30]. However, PEDD biopsy and CT-guided biopsies revealed an equal prevalence [50% (9/18) vs. 44.4% (8/18)] to identify *S. aureus* infections. Furthermore, cytokines are also possible markers for diagnosis. For example, IL-17 plays an important role in the inflammatory process in ankylosing spondylitis [31]. To predict the clinical outcome of patients with bacterial pyogenic vertebral osteomyelitis, an erythrocyte sedimentation rate (ESR) over 55 mm/h and a C-reactive protein (CRP) value of 2.75 mg/dL at the fourth week are useful markers [6]. As an important regulatory gene, *agr* (and its protein) regulates the expression of cell surface and extracellular virulence factors [32] [33]; most human MRSA isolates are of the *agr* 1 type [34]. In this study, *agr*1 was found in MRSA SCC*mec* IIIA MDR isolates 2, 3, and 6 with higher resistance to carbapenem, CC and ERY than other *agr* types and SCC*mec* IV MRSA (**Table 2**), demonstrating that MRSA SCC*mec* IIIA isolates may be more difficult to treat. However, with suitable antibiotic treatment, patients can completely recover from pyogenic thoracic spondylodiscitis with an epidural abscess caused by *S. aureus* infection [35].

In general, almost all of the *S. aureus* isolates lacked the toxin genes *tst*, *eta* and *etb* (**Table 1** and **Table 3**). However, two important virulence genes that were identified in all of the isolates were leukocidin genes, which encode bi-component toxins including PVL (LukS-PV + LukF-PV), LukM (LukM + LukF'-PV) and LukED (LukE + LukD) [29] [36]. Here, we determined that the ST959, ST239, and ST15 isolates carried genes *pvl* and a plasmid, whereas the ST30 and ST59 isolates carried genes *lukED* and *hlg*-2 or *hlg* and lacked a plasmid and the hemolysin gene (**Table 3**). Additionally, PVL, staphylococcal protein A and coagulase inhibited proliferation and induced apoptosis of osteoblasts [37]. A recent finding has demonstrated that α-toxin (Hla) is an important virulence factor that causes membrane damage and induces apoptosis of human peripheral blood mononuclear cells (PBMCs) during USA300 infection [38].

In the present study, all six clinical isolates caused more necrosis than apoptosis (**Table 4**). Regardless of MRSA or MSSA, SCC*mec* type, and hemolysin genes, isolates 4, 5, and 6 caused more cell death of the human alveolar basal epithelial cell line A549, with a ratio of necrosis over late apoptosis equaling more than 2.84. Isolates 5 and 6 were SCC*mec* IV ST30 and SCC*mec* IIIA-like MRSAs that both carry the PVL gene, not LukED, whereas isolate 4 was MSSA and the only strain to carry *tst*. Therefore, the hemolysin genes, PVL, and *tst* may be important virulence factors for *S. aureus* to cause spondylitis.

5. Conclusion

In conclusion, *M. tuberculosis* and *S. aureus* were the major pathogens to cause infectious spondylitis. MRSA and MSSA differed with respect to the infection sites. All six *S. aureus* strains caused more necrosis than apoptosis. Regardless of MRSA and MSSA, the strains with *pvl* or *tst* caused more cell necrosis.

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