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Highlights

- In children and adults, PVL toxin could promote hematogenous osteomyelitis (OM)
- Particular lineages as MSSA ST398 and PVL-positive lineages are associated with OM
- The most frequent clones causing OM were the same described in HA or CA infections

Staphylococcus aureus clones causing osteomyelitis; a literature review (2000-2020)

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Abstract

Objectives *Staphylococcus aureus* is the most common causative organism of osteomyelitis (OM). Nevertheless, the molecular epidemiology of *S. aureus* causing OM remains ill-defined. This study aims to address the global epidemiology of *S. aureus* clones from OM patients.

Methods Literature databases were searched for studies reporting the molecular typing of *S. aureus* involved in OM, published between January 1, 2000 and July 29, 2020. Data from 32 articles that fulfilled inclusion criteria were analyzed for year of publication, country of patients, methicillin-susceptibility, and genotypic characteristics of *S. aureus* isolates.

Results Pandemic clones CC5, CC8, CC22, CC30 and CC45 were the most common in OM. The distribution of clones greatly differed among studies due to the local epidemiology of *S. aureus* and to the MSSA heterogeneity. PVL-positive MRSA clones belonging to ST80/CC80 and ST8/CC8/USA300 were the most common among paediatric patients in Europe and USA; greater variability was observed in the adult population. In Europe, MRSA belonged to PVL-negative CC5, CC8 and CC22 indicating a nosocomial origin of infections; in Asia the PVL-positive ST59/CC59 MRSA was the

most frequent. PVL-positive clones were often detected in hematogenous OM in children and adults. Although MSSA were polyclonal, the PVL-negative ST398/CC398 MSSA was the most prevalent clone in diabetic foot OM.

Conclusions All major *S. aureus* clones circulating in both hospital and community settings appear to be capable of causing OM. Future studies reporting molecular typing and genomic data will provide more insights into the epidemiology and pathobiology of *S. aureus* clones causing OM.

Keywords: *Staphylococcus aureus;* osteomyelitis; MRSA; molecular typing; clones; molecular epidemiology.

1. Introduction

Osteomyelitis (OM) is an infective and inflammatory process of the bone, which can progress to osteonecrosis and bone destruction [1]. Several aspects contribute to the complexity of OM, principally the heterogeneity of the clinical presentation and the pathophysiology of the disease. Based on the source of infection and etiology, Waldvogel [1] classified OM in three categories: *i*) haematogenous OM; *ii*) contiguous OM, *i.e.* OM caused by the contiguous spread of infection from an exogenous source; *iii*) vascular insufficiency OM. Atypical feature of OM is the bimodal age distribution with a peak of incidence in children under 5 and adults over 50 years of age [2]. Haematogenous OM is the most frequent type of OM in children, accounting for up to 80% of the cases, and affecting mostly long bones, while in adults haematogenous OM affects most frequently spinal bones [3]. In younger adults, OM is often related to trauma or surgery. In older adults, contiguous OM is the most common type of OM, being frequently associated with orthopaedic surgery or infected diabetic ulcers, mainly in the lower extremities [3]. All types of microorganism, including bacteria, viruses, parasites, and fungi may cause OM, although bacteria, and particularly Gram-positive bacteria, are the most frequent. *Staphylococcus aureus* is responsible for up to 60% of all OM cases, followed by coagulase-negative staphylococci, such as *Staphylococcus epidermidis*, and *Streptococcus* species. Among Gram-negative bacteria, *Pseudomonas aeruginosa* and a few emerging pathogens, such as the fastidious organism *Kingella kingae*, predominate [3, 4]. Many studies indicate a severe prognosis for OM due to *S. aureus* which may be related to the broad virulence armamentarium of this organism and the immunopathological reaction in response to the bacterial toxins [5, 6].

S. aureus possesses a large arsenal of virulence factors. In particular, *S. aureus* expresses several virulence factors that allow the interaction of the bacterial cells with the extracellular bone matrix, such as adhesins or MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) [6]. *S. aureus* can also produce biofilm, invades osteoblasts and survives intracellularly by conversion to an intracellular status known as small-colony variant [6]. The peculiar characteristics of the infection make the management of *S. aureus* OM quite challenging, since it requires prolonged antibiotic therapy and is frequently associated with relapses. The choice of an effective antibiotic therapy can be difficult, especially for methicillin-resistant *S. aureus* (MRSA) which is frequently multi-drug resistant.

Consequently, OM due to MRSA is associated with more severe clinical outcome and higher frequency of long-term sequelae, as compared with OM due to methicillin-sensitive *Staphylococcus aureus* (MSSA) [7].

MRSA causing infections in humans can be classified into three broad categories on the basis of epidemiologic and microbiologic criteria: healthcare-associated (HA), community-associated (CA), and livestock-associated (LA) MRSA [8]. In the present time these distinctions are blurred due to the osmosis of isolates from one setting to the other. Each clone or even strain is endowed with distinct antibiotic-resistance characteristics and/or virulence traits [8]. Sequence-based methods as multilocus sequence typing (MLST) and *spa* typing, extensively used to differentiate *S. aureus* clones, were recently replaced by more informative whole genome sequencing (WGS) data [9]. However, information regarding the molecular types of *S. aureus* responsible for OM is very limited, since there is a tendency to classify the strains based on their epidemiology, history of the patients and presence of the Panton–Valentine leucocidin (PVL) toxin, without molecular typing data. To provide an updated overview of the major clones of *S. aureus* causing of OM and OM-related diseases in humans worldwide, the available literature has critically been reviewed.

2. Methods

2.1 Literature review and information sources

A literature review was conducted to identify studies reporting the molecular typing of *S. aureus* from bone and joint infections, including osteomyelitis, arthritis, and implant-related infections. The literature was selected by searching in the title, abstract and controlled terms of four databases: MEDLINE, BIOSIS, EMBASE, and SCISEARCH.

2.2 Inclusion and exclusion criteria

Single and multicentre studies and case reports were included if they: *i*) reported the molecular typing as Sequence Type (ST) or Clonal Complex (CC) of *S. aureus* isolates from patients diagnosed for OM or bone and joint infection; ii) were published between January 1, 2000 and July 29 2020. Studies were excluded if they classified *S. aureus* as community-associated MRSA/MSSA (CA-MRSA/MSSA) or hospital-associated MRSA/MSSA (HA-MRSA/MSSA) or livestock-associated MRSA/MSSA (LA-MRSA/MSSA) only based on the clinical history of the patients without molecular typing. No restriction was made based upon language and articles regarding animals were excluded.

2.3 Search strategy

Literature searches were performed using the search terms: "*Staphylococcus aureus*" and "osteomyelitis" and/or "bone and joint infections", and/or "arthritis", and/or "implant-related infection" and "typing" or "clone" or "MLST" or "*spa* type" or "community-associated MRSA" or "CA-MRSA" or "community-associated MSSA" or "CA-MSSA" or "hospital-associated MRSA" or "HA-MRSA"or "hospital-associated MSSA" or "HA-MSSA" or "livestock-associated MRSA" or "LA-MRSA" or "livestock-associated MSSA" or "LA-MSSA".

2.4 Data extraction and management

The following data, if available, were extracted from each article: *i*) year of publication; *ii*) country of the study; *iii*) study design; *iv*) study size; *v*) methicillin-resistance (MSSA *vs.* MRSA); *vi*) age of patients; *vii*) genotypic characteristics of *S. aureus* isolates (*spa* type, ST, CC); *viii*) presence of PVL toxin.

3. Results

3.1 The review process

The literature searches produced 493 articles. Duplicates and articles that did not meet the inclusion criteria were excluded. A screening of the abstracts was performed to select relevant articles, and 32 fulfilled the inclusion criteria, comprising the molecular typing of the isolates (Figure 1).

3.2 Selected literature

Out of the 32 selected papers, 11 (34%) were single-centre studies, 6 (19%), multicentre studies and 15 (47%) case reports (Figure 1). The single-centre (SC) and multicentre (MC) studies reported the characteristics of different isolates (from 12 to 958), most of which originating from OM or bone and joint infections. Twelve studies (38%) were conducted in patients under 18 years of age, 12 (37.5%) in adults; for 6 studies (19%) the age of the patients was not available, and two studies includes patients of all ages (Tables 1 and 2).

3.4 S. aureus clones isolated from OM

3.4.1 Paediatric population

Four studies were conducted among paediatric patients in Greece, Tunisia, USA and Korea, involving children with OM aged 1-18 years [10–13]. The studies conducted in Greece, Tunisia and Korea included bone and joint infections caused by both MSSA and MRSA, while only MRSA were included in the study from USA. The peculiarity of the studies conducted in Greece, Tunisia and USA was that in each country a single clone was prevalent among MRSA and carried the PVL toxin encoding genes. In the multicentre (MC) study from Greece, MRSA represented 38.2% of the isolates and the most common MRSA clone was the PVL-positive ST80/CC80 (92.3%) while MSSA isolates showed polyclonality [10] (Table 1). In the study from Tunisia, MRSA accounted for 41.7% of the isolates, all belonging to the PVL-positive ST728/CC80 clone [11] while in USA all the MRSA isolates belonged to the PVL-positive ST8/CC8/USA300 pandemic clone. In the study from Korea, MRSA represented 23.1% of the isolates and ST30/CC30 was the predominant clone (34.6%) in both MRSA and MSSA; no data were provided about the carriage of PVL genes in Korean isolate [13] (Table 1). Considering the 8 case reports involving children, 5 (62.5%) described mostly hematogenous OM caused by pandemic PVL-positive strains [14–21] (Table 2).

3.4.2 Adult population

Five studies were conducted in the adult population, 4 in France and 1 in Germany (Table 1). In the first French MC study, MSSA and MRSA were isolated from patients suffering from diabetic foot OM [22]. The most common clone was the PVL-negative ST398/CC398, which accounted for 46.0% of MSSA isolates. MRSA represented only 16.0% of the isolates and belonged to CC8, CC5, and CC22 (Table 1). Due to the epidemiological importance of these clones in bone infections, two other French studies screened a large collection of *S. aureus* isolated from bone and joint infection (485 and 958 respectively) and observed the CC398 in more than 14% of the MSSA [23,24]. In one of these two papers, Valour *et al.* [23] also carried out a parallel SC study including 75 MSSA with the most common clones belonging to CC30, CC5, CC45 and CC398. In another French study, only one isolate belonging to CC398 was detected, while the most common clones were CC30, CC5, and CC8 [25]. In the German SC study, isolates were obtained from patients with haematogenous OM or orthopaedic prosthetic infections [26]. In haematogenous OM, the majority of MSSA isolates and the single MRSA belonged to CC22 whereas in orthopaedic prosthetic infections MSSA belonged mostly to CC7 (40.0%) and MRSA to CC8 and CC5 (Table 1). Regarding the 7 case reports of OM in adults, 6 were due to MRSA [27–33] (Table 2). PVL-positive MRSA were reported from haematogenous OM and arthritis and included the pandemic clones ST8/CC8/USA300, ST30/CC30 and the "Bengal Bay clone" ST772/CC1, commonly circulating in the South-East Asia (Table 2). The case described by Nakaminami *et al.* [33] reported for the first time an invasive infection caused by ST1232/CC398 MRSA, a single locus variant of ST398, in a patient who had not had previous animal contact (Table 2).

3.4.3 Studies with patients of all ages or unknown age

Eight studies involving patients with bone and joint infections were conducted in Europe, South America and Asia [34–41] (Table 1). Unfortunately, in 6 out of 8 studies the age of patients was unavailable, in the other two studies patients with different ages were included. In Germany, CC8, CC45 and CC30 were the most prevalent clones among MSSA while MRSA accounted for only 5.9% of the isolates mostly belonging to CC5 [34] (Table 1). In the MC study conducted in Switzerland and France, MRSA accounted for 25.7% of the isolates and CC5 and CC8 were identified. The MSSA isolates were mostly represented by CC30, CC45, CC59 and CC5. The prevalence of PVL-positive strains in MSSA and MRSA was low (1.6%). In Italy, the most frequent clone detected among MSSA from orthopaedic implant-related infections was CC30 and the PVL was detected in one isolate only [36]. Two large MC studies were performed in South America. In Argentina, high MRSA rate (45.7 %) was reported; the most frequent CC in MSSA and MRSA isolates were CC5, CC97, CC1, CC8 and CC30, and PVL encoding genes were detected in 25.5% of the isolates [37] (Table 1). In the second South American study, only MSSA isolates were considered, and the most common clones were CC5, CC8, CC30 and CC45; no data were available about the carriage of PVL toxin genes [38] (Table 1). In one SC study carried out in China, MRSA represented 15.0% of the isolates [39]. The most frequent clones among MRSA

and MSSA belonged to CC1 followed by CC59, the latter being frequent in Asia. The PVL toxin encoding genes were detected in 28.3% of the isolates (Table 1). A SC study including only MRSA from OM was conducted in Taiwan in patients aged from 15 to 98 years old (mean 60) [40]. Interestingly, a high rate of PVL positive strains (56%) was detected and the most representative clones belonged to CC8 and CC59. The Norwegian SC study included patients of all ages and the most frequent clones belonged to CC45 and CC30 while the single MRSA belonged to CC5; no isolates carried the PVL toxin encoding genes [41].

4. Discussion

Despite the importance of *S. aureus* in bone and joint infections, the number of studies that reported the molecular characteristics of *S. aureus* in OM isolates is surprisingly low. A substantial number of publications on OM was excluded from the review due to the lack of molecular characterization (ST, CC, or *spa* types) which is fundamental for understanding the epidemiology and circulation of clones. Nowadays, the epidemiological classification in HA and CA clones has lost its value, as in the last decades there has been a migration of these clones between community and hospital environments [42]. LA lineages as well have become cause of hospital infections, in patients without previous contact with farm animals [43]. Therefore, in descriptive studies, molecular typing is vital for understanding the genetic background and tracing the epidemiology of isolates from both hospital and the community settings.

In this review, the most frequent OM type described in paediatric patients was haematogenous OM. MRSA isolates were more frequently detected among children than from the adult population. MRSA in paediatric patients ranged from 38.2 to 41.7% of the isolates while in adult patients from 7.7 to 16.7% of the isolates. PVL-positive strains among paediatric patients were detected in 96.2 to 100% of the MRSA isolates. Indeed, most of the strains isolated from paediatric patients belonged to CA-MRSA lineages that produce the PVL toxin. In Greece and Tunisia, almost half of the isolates from paediatric patients belonged to the CC80 lineage which includes the PVL-positive "European clone" ST80, the most common CA-MRSA in Europe [10,11]. Although limited to a single study, the PVL-positive CA-MRSA ST8/CC8/USA300 was the only clone found among children with OM in the USA [12]. The PVL toxin is a two-component toxin acting on cellular targets such as polymorphonuclear leukocytes, monocytes and macrophages leading to cell destruction [2], and is therefore considered an important virulence factor, especially in necrotizing infections [44]. PVL-producing strains have been linked with aggressive manifestations, *e.g.* severe sepsis, septic arthritis, multifocal OM involving multiple bones, and intraosseous and intramuscular abscesses [2,45]. PVL-positive strains are considered quite rare in *S. aureus* musculoskeletal infections [44], although in children with haematogenous OM PVL-positive strains were frequently detected [10–12, 46, 47]. It is unclear why paediatric patients are so

frequently affected by PVL-positive strains. One possible explanation is that CA-MRSA carrying PVL genes mostly affect young and healthy people in the community [48].

Regarding adult patients, OM was mainly due to a contiguous spread from infected or contaminated body site to the bone tissue as the result of trauma, orthopaedic surgery, implant-related infections, or diabetic foot infections. Different from studies in paediatric patients, in adults PVL-positive strains were rarely detected, and their frequency ranged from 0 to 2.7% for MSSA and from 0 to 14.3% for MRSA. Only in one study from Taiwan, involving patients from 15 to 98 years, 56% of MRSA isolates carried the PVL genes and belonged to CA-MRSA ST59/CC59 known as the "Taiwan Clone" [40]. Interestingly, also the adult cases of haematogenous OM described in some case reports were associated with PVL-positive CA-MRSA suggesting that the PVL toxin could favour infection of the bone through haematogenous dissemination of *S. aureus.* In most of the adult studies, the MRSA lineages detected in OM were CC5, CC8 and CC22, which are frequently isolated in hospital settings, suggesting a link between OM in adults and a nosocomial origin of the strain.

Different from MRSA, the MSSA isolates from OM were very heterogeneous, belonging to a variety of different lineages. One exception was represented by the MSSA ST398/CC398 that was the most frequent clone detected among adult patients affected by OM in studies conducted in France [22–24]. ST398 was first described as a livestock-associated MRSA, and many studies reported infections with LA-MRSA ST398 worldwide [49]. The human-associated MSSA ST398 diverged from the livestock lineage approximately 40 years ago [50], and has recently been reported as a cause of invasive infections with a high risk of mortality in patients without livestock contact, in both community and hospital settings, in various parts of the world, including Europe, China, and the United States [50–52]. The high prevalence of this clone in OM in France probably reflects the local epidemiology, since the presence of MSSA ST398/CC398 in invasive infections was frequently documented in France during the last years [53, 54].

Despite the limitations of this review, consisting in the small number of eligible studies, and the heterogeneity of the data analysed, some conclusions can be drawn. In general, the MRSA clones identified in the various studies, both in children and in adults, reflect the local epidemiology of *S. aureus* infections: in children the majority of the isolates causing OM are PVL-positive CA-MRSA. CC80 is prevalent in Europe, while ST8/CC8/USA300 is prevalent in the USA. In adults, OM are mainly caused by the major pandemic HA-MRSA lineages such as CC5, CC8, and CC22, with the exception of the PVL-positive CA-MRSA CC59 circulating in Asia. In children, as well as in adults, PVL-positive strains are frequently detected from cases of hematogenous OM suggesting a role for PVL in promoting hematogenous OM. Generally, MSSA causing OM in both children and adults belonged to heterogeneous genotypes and, with few exceptions (e.g. MSSA ST398), no prevalent lineages were identified.

The most frequent clones causing bone infections were the same pandemic clones described in HA or CA infections [49, 55–57]. All major *S. aureus* clones widespread in both hospital and community settings appear to be capable of causing bone and joint infections. However, it can be hypothesized that particular lineages (*e.g.* PVL-positive lineages, MSSA ST398) possess additional virulence factors that facilitate bone infection. Therefore, it is mandatory to define the genotype of *S. aureus* causing OM by applying up-to-date genomic approaches, primarily WGS, in order to trace more precisely the epidemiology of isolates and highlight genetic trait(s) favouring the adhesion and invasion of the bone tissues (adhesins, immuno-evasion factors and biofilm formation genes), as well as tissue damage (toxins). Improving the knowledge about OM pathogenesis and gaining more insights into the genetic background of *S. aureus* causing OM could contribute to adopt more effective prevention and treatment strategies for patients affected by this serious infection.

Authors' contributions

AP, PV, and FPA conceived and designed the study. FPA acquired the data and drafted the manuscript. FPA, AP, PV and MM analysed and interpreted the data. MM, MP and MDG participate in drafting the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of interests

The authors declare that they have no conflict of interest.

Ethical approval

Not required

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Figure caption

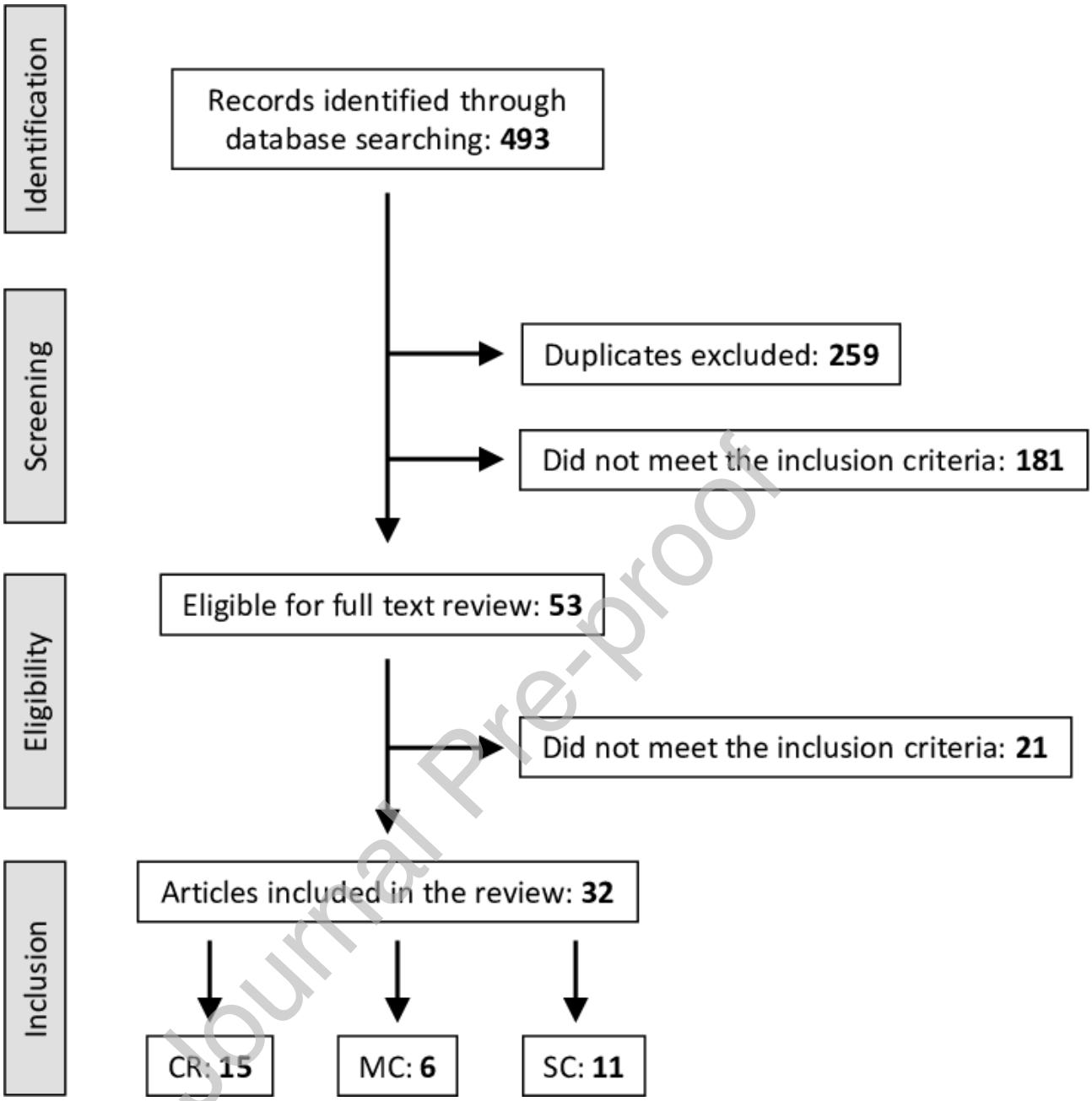

Fig. 1. Literature search and selection. CR, case reports; MC, multicentre studies; SC, single-centre studies.

Table 1. Characteristics of *Staphylococcus aureus* strains isolated from patients with osteomyelitis in clinical studies

Infection type	Patient age (years range)	Study type	Country	No. of *S. aureus* isolates[a]	MSSA/MRSA: no. of isolates (%)	MSSA/MRSA: ST[b]/CC[c] (% of isolates)	MSSA/MRSA: no. of PVL-positive isolates (%)	Ref.
Children								
Osteoarticular infection	<1-16	MC	Greece	68	MSSA: 42 (61.8) MRSA: 26 (38.2)	MSSA: polyclonal CCs MRSA: ST80/CC80 (92.3), ST217, ST377	MSSA: 2 (4.8) MRSA: 25 (96.2)	[10]
Osteomyelitis	<1-14	SC	Tunisia	12	MSSA: 7 (58.3) MRSA: 5 (41.7)	MSSA: ST772/CC1 (42.8), ST30/CC30, ST728/CC80, ST1468, ST1469 MRSA: ST728/CC80 (100)	MSSA: 5 (71.4) MRSA: 5 (100)	[11]
Osteomyelitis	1-13	SC	United States	12	MRSA: 12 (100)	MRSA: ST8/CC8/USA300 (100)	MRSA: 12 (100)	[12]
Bone and joint infection	<1-18	SC	Korea	26	MSSA: 20 (77.0) MRSA: 6 (23.1)	MSSA/MRSA[d]: ST30/CC30 (34.6), ST72/CC8 (26.9), ST5/CC5 (7.7), ST6/CC5 (7.7), ST188/CC1 (7.7), ST1/CC1, ST121, ST398/CC398, ST7	NA	[13]
Adults								
Diabetic foot osteomyelitis	38-85	MC	France	81	MSSA: 68 (81.0) MRSA: 13 (16.0)	MSSA: CC398 (46.0), CC59 (8.8), CC30 (5.4), CC5 (5.9), CC7 (5.9), others (26.5) MRSA: CC8 (69.2), CC5 (23.1), CC22	MSSA: 0[e] MRSA: 0[e]	[22]
Bone and joint infection	NA	MC	France	485	MSSA: 485 (100)	MSSA: CC398 (14.0); others (86.0)	NA	[23]
	52.7[f]	SC		75	MSSA: 75 (100)	MSSA: CC30 (16.0), CC5 (13.3), CC45 (12.0%), CC398 (10.7); others (48.0)	NA	
Bone and joint infections	62.2[g]	SC	France	958	MSSA: 821 (85.6) MRSA: 137 (14.3)	MSSA: CC398 (14.6); others (85.4) MRSA: CC398 (2.9); others (97.1)	NA	[24]
Prosthetic joint infection	21-96	SC	France	56	MSSA/MRSA: NA	MSSA/MRSA[d]: CC30 (20.0), CC5 (20.0), CC8 (15.0), CC45 (12.0), CC15 (7.0), others (26.0)	NA	[25]
Haematogenous osteomyelitis	47-96	SC	Germany	13	MSSA: 12 (92.3) MRSA: 1 (7.7)	MSSA: CC22 (25.0), CC15 (16.7), CC1, CC6, CC8, CC25, CC45, CC101, CC121 MRSA: CC22[c]	MSSA: 0 MRSA: 0	[26]
Orthopaedic prosthetic infection	33-85			12	MSSA: 10 (83.3) MRSA: 2 (16.7)	MSSA: CC7 (40.0), CC5 (20.0), CC8 (20.0), CC6, CC30 MRSA: ST239/CC8, CC5	MSSA: 3 (2.7) MRSA: 1 (14.3)	
Age unavailable								
Bone and joint infections	NA	SC	Germany	119	MSSA: 112 (94.1) MRSA: 7 (5.9)	MSSA: CC8 (20.5), CC45 (17.9), CC30 (13.4), CC101 (8.0), CC25 (7.1), CC15 (6.3), CC12 (5.4), others (21.4) MRSA: CC5 (57.1), CC22, CC45, CC80	MSSA: 3 (2.7) MRSA: 1 (14.3)	[34]
Musculoskeletal infections[h]	NA	MC	Switzerland, France	109	MSSA: 81 (74.3) MRSA: 28 (25.7)	MSSA: CC30 (24.7), CC45 (16.0), CC59 (12.3), CC5 (12.3), CC1 (7.4), ST398/CC398 (7.4), ST1954 (7.4), ST217 (6.2), CC8 (6.2) MRSA: CC5 (71.4), CC8 (28.6)	MSSA/MRSA[d]: 4 (1.6)	[35]
Orthopaedic implant related infections	NA	SC	Italy	27	MSSA: 27 (100)	MSSA: CC30 (100)	MSSA: 1 (3.7)	[36]
Osteomyelitis	NA	MC	Argentina	94	MSSA: 51 (54.3) MRSA: 43 (45.7)	MSSA/MRSA[d]: CC5 (28.7), CC97 (19.1), CC1 (14.9), CC8 (12.8), CC30 (8.5), others (16.0)	MSSA/MRSA[d]: 24 (25.5)	[37]
Osteomyelitis	NA	MC	Colombia, Ecuador, Peru, Venezuela	68	MSSA: 68 (100)	MSSA[i]: ST5/CC5, ST8/CC8, ST30/CC30, ST45/CC45	NA	[38]
Osteomyelitis	NA	SC	China	60	MSSA: 51 (85.0) MRSA: 9 (15.0)	MSSA/MRSA[d]: ST188/CC1 (18.3), ST59/CC59 (15.0), others (66.7)	MSSA/MRSA[d]: 17 (28.3)	[39]

Children and adults								
Osteomyelitis	15-98	SC	Taiwan	115	MRSA: 115 (100)	MRSA: ST59/CC59 (32.0), ST239/CC8 (24.0) ST8/CC8 (21.0), ST45/CC45 (6.0); others (17.0)	MRSA: 64 (56.0)	[40]
Osteomyelitis/ arthritis[j]	0-96	SC	Norway	12	MSSA: 11 (91.7) MRSA: 1 (8.3)	MSSA: CC45 (36.4), CC30 (18.2), others (45.5) MRSA: CC5[c]	NA	[41]

MC, multicentre; NA, not available; ST, sequence type; CC, clonal complex; PVL, Panton-Valentine leucocidin; SC, single-centre.
[a]Number of characterized isolates for the type of infection, reported in the publication.
[b]ST is reported where available
[c]CC percentage is not indicated when only one strain is present.
[d]Clones were not separated for MSSA and MRSA.
[e]PVL was tested only for CC398.
[f]Mean of age reported in the study.
[g]Mean of age of the patients with CC398 (62.2); mean age of patients with other CCs different from CC398 (64.3).
[h]Musculoskeletal infections include orthopaedic implant related infections and non-implant related infections.
[i]Percentage not available for reported clones.
[j]Strains obtained from patients with clinical parameters attributable to osteomyelitis or arthritis.

Table 2. Characteristics of *Staphylococcus aureus* strains isolated from patients with osteomyelitis (case reports)

Infection type	Patient(s) age (years)	Country	Methicillin susceptibility	ST/CC	PVL	Ref.
Children						
Osteomyelitis	17	Japan	MRSA	ST30/CC30	+	[14]
Haematogenous osteomyelitis	10	Brazil	MRSA	ST30/CC30	+	[15]
Haematogenous osteomyelitis	<1	Japan	MRSA	ST8/CC8/USA300	+	[16]
Haematogenous and trauma-related osteomyelitis[a]	9, 10	Chile	MRSA	ST8/CC8 (2)	-	[17]
Haematogenous osteomyelitis	12	China	MRSA	ST59/CC59	+	[18]
Osteomyelitis	15	Taiwan	MSSA	ST59/CC59	-	[19]
Haematogenous osteomyelitis	12	Brazil	MSSA	ST2104/CC25	+	[20]
Haematogenous osteomyelitis	<1	United Kingdom	MRSA	ST22/CC22	-	[21]
Adults						
Haematogenous osteomyelitis [a]	27, 37	Germany	MRSA	ST8/CC8/USA300 (2)	+	[27]
Haematogenous osteomyelitis	19	India	MRSA	ST772/CC1	+	[28]
Haematogenous osteomyelitis	28	Malaysia	MRSA	ST30/CC30	+	[29]
Osteomyelitis	57	Singapore	MRSA	ST571/CC8	-	[30]
Osteomyelitis	48	Spain	MSSA	ST120/CC121[b]	-	[31]
Haematogenous osteomyelitis	61	Denmark	MRSA	ST398/CC398	-	[32]
Arthritis	74	Japan	MRSA	ST1232/CC398	+	[33]

ST, sequence type; CC, clonal complex; PVL, Panton-Valentine leucocidin; +, positive; -, negative.
[a]Two different cases.
[b]Isolates with different ST included in CC121 were also reported.