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Staphylococcus aureus Genetic Lineages Found in Urban Effluents and River Water

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Abstract

Methicillin resistant and susceptible *Staphylococcus aureus* (MRSA and MSSA respectively) remain a public health concern as human pathogens. Presence of MRSA and MSSA in river water and urban effluents was studied to analyze the *S. aureus* population and determine the genetic diversity and predominant genotypes obtained by *spa* types and MLST on each ecological niche. MRSA proportion in urban effluents was higher than in river water (P<0.05). According to the Simpson's Index of Diversity based on *spa* types, MSSA isolates were more diverse than MRSA isolates (P<0.05). Predominant *spa* types and STs detected in MSSA river water isolates were different from those found in urban effluents. In the MRSA population, ST125-t067 was the predominant genotype detected in both urban effluents (67.6%) and river water (82.4%). Overall, the MSSA and MRSA lineages most frequently found in river water and urban effluents were human associated clones (ST125-t067, ST5-t002; ST22-t032, ST30-t012 and ST15-t084). These results show the potential role of water in the *S. aureus* maintenance and dissemination. Association of isolates from the river with human ones could be reflecting the effect of anthropogenic activities in the ecosystems, which highlights the need to evaluate the circulation of pathogens in the environment via water.

Keywords: *Staphylococcus aureus*; Urban effluents; River water; *Spa* typing; MLST

Introduction

Methicillin resistant and methicillin susceptible *Staphylococcus aureus* (MRSA and MSSA) remains a public health concern as human pathogens [1]. Different genetic lineages have been described as Hospital Associated-MRSA (HA-MRSA), Community-Associated-MRSA (CA-MRSA) and Livestock Associated-MRSA (LA-MRSA). Infections caused by HA-MRSA isolates are normally related to risk factors such as hospitalization, surgery or indwelling medical devices [2]. CA-MRSA affects to otherwise healthy people and infections have been linked to the presence of the toxin Panton-Valentine leukocidin or PVL [2]. Finally, LA-MRSA has been considered an occupational risk although its frequency of isolation is increasing in countries with low prevalence of MRSA [3]. Genetic differentiation between these groups is getting more complicated due to the incidence of HA-MRSA in the community and *vice versa* and due to the transmission of MRSA between humans and animals [4]. Direct contact was pointed out as the most feasible transmission route of *S. aureus* [3]. However, colonized individuals might discharge bacteria into urban effluents and recreational water [5,6]. Wastewater treatment plants have been described as reservoirs for MRSA, and hypothetically, participate in their dissemination through sewage treatment plant effluents, as part of the *S. aureus* population might survive the wastewater treatments [6-9]. Moreover, the presence of MRSA in river water [10] points out the potential role of water in the dissemination of MRSA, and in consequence, into associated environments [8, 9, 11]. These facts led us to study the presence of MSSA and MRSA in urban effluents and river water to assess the genetic diversity and predominant genotypes within each ecological niche.

Experimental Section

Samples origin

One sample of urban effluents was taken in July 2011 in a sewage plant that gathers wastewater from several urban collectors (untreated wastewater) in an urban nucleus with 3.2 million people (http://www.ine.es/SID/Informe.do). One river water sample was taken in September 2012 in the countryside, downstream the municipal term of a city with 8,392 people (http://www.ine.es/SID/Informe.do).

Isolation and characterization

Both samples were divided into sub-samples and processed separately (n=100 subsamples per sample). Each sub-sample (1 mL) was cultured on 9 mL of Muller-Hinton broth (6.5% NaCl, Oxoid) and incubated at 37°C for 16-20 h. One mL was then transferred to 9 mL tryptone soy broth (Oxoid) with cefoxitin (3.5 mg/L, Sigma–Aldrich) and aztreonam (75 mg/L, Sigma–Aldrich) and incubated at 37°C for 16–20 h. Finally, 25 µL were streaked onto Brilliance MRSA plates (Oxoid) and incubated for 24–48 h at 37°C [10]. Denim blue colonies (one per subsample) were confirmed as MRSA (*mecA* or *mecC* positive) by PCR [12]. In parallel, 100 µL of incubated Muller-Hinton broth (6.5% NaCl) were cultured onto Baird Parker (BP) agar with Rabbit Plasma Fibrinogen (bioMerieux) and incubated at 37°C during 24-48 h. Black colonies coagulase-positive

(one per subsample) were selected as potential *S. aureus* and confirmed as MRSA (*mecA* or *mecC* positive) or MSSA (*mecA* and *mecC* negative) as described above. Confirmed *S. aureus* were characterized by *spa* typing sequencing the variable fragment of protein A [12], and *spa* types were analysed by the minimal Spanning tree algorithm (Bionumerics 6.0). Simpson's Index of Diversity (SID) and Jackknife pseudo-values (CI: 95%) were used to estimate the genetic diversity of *S. aureus* isolates based on *spa* types (Figure 2; http://darwin.phyloviz.net/ComparingPartitions/index.php?link=Tool). Multilocus Sequence Typing (MLST) was performed to at least one isolate per *spa* type and isolation route (n=103) to obtain the sequence types (STs) according to the protocol described before [13]. Detection of Panton–Valentine leukocidin (PVL) was also carried out [12].

Statistical analysis

Fisher's exact test (SPSS 20) was calculated to analyze the relationship between the type of sample (urban effluents or river water) and the presence of MRSA and between the type of sample and the most frequent *spa* types and STs in the collection (n>5 isolates).

Results and Discussion

MRSA protocol detected 96 MRSA isolates out of 100 subsamples in urban effluents, meanwhile only 33/100 MRSA in river water (Table 1). All isolates obtained by this protocol were *mec*A-MRSA. On the Baird Parker protocol, most of *S. aureus* isolates were MSSA (Table 1), but some MRSA were also detected (5 isolates *mec*A-MRSA and 1 isolate *mecC*-MRSA in urban effluents and 1 *mec*A-MRSA in river water). The low detection rates of *mecC*-MRSA compared with *mec*A-MRSA is in agreement with other studies [13-16]. However, the detection of *mecC*-MRSA in water effluents is of interest considering the potential for zoonotic transmission [17] and wildlife-environmental interactions of *mecC*-positive MRSA [10].

Only one isolate MSSA from river water was positive to PVL (ST737-*spa* type t4801). Some studies described that PVL is increasing in the south of Europe and in some areas in *Spa*in, but those are referred mainly to ST8 and ST80 [18,19], STs whose isolation frequency in our study (Table 1) was low (ST8) or undetected (ST80).

A higher proportion of MRSA isolates was detected in urban effluents (102/169; 60.4%) than in river water (34/115; 29.6%), differences statistically significant ($P<0.05$). This higher frequency of isolation of MRSA in urban effluents compared with the river water might be related to the higher concentration of antimicrobial resistant bacteria in wastewater and the population density in the area of sampling [20,21].

Isolates were grouped in 81 different *spa* types (Figure 1) and 42 STs, with 12 *spa* types and 14 STs being common to both environments (Table 1). Ten new *spa* types and 7 new STs were firstly described in this study

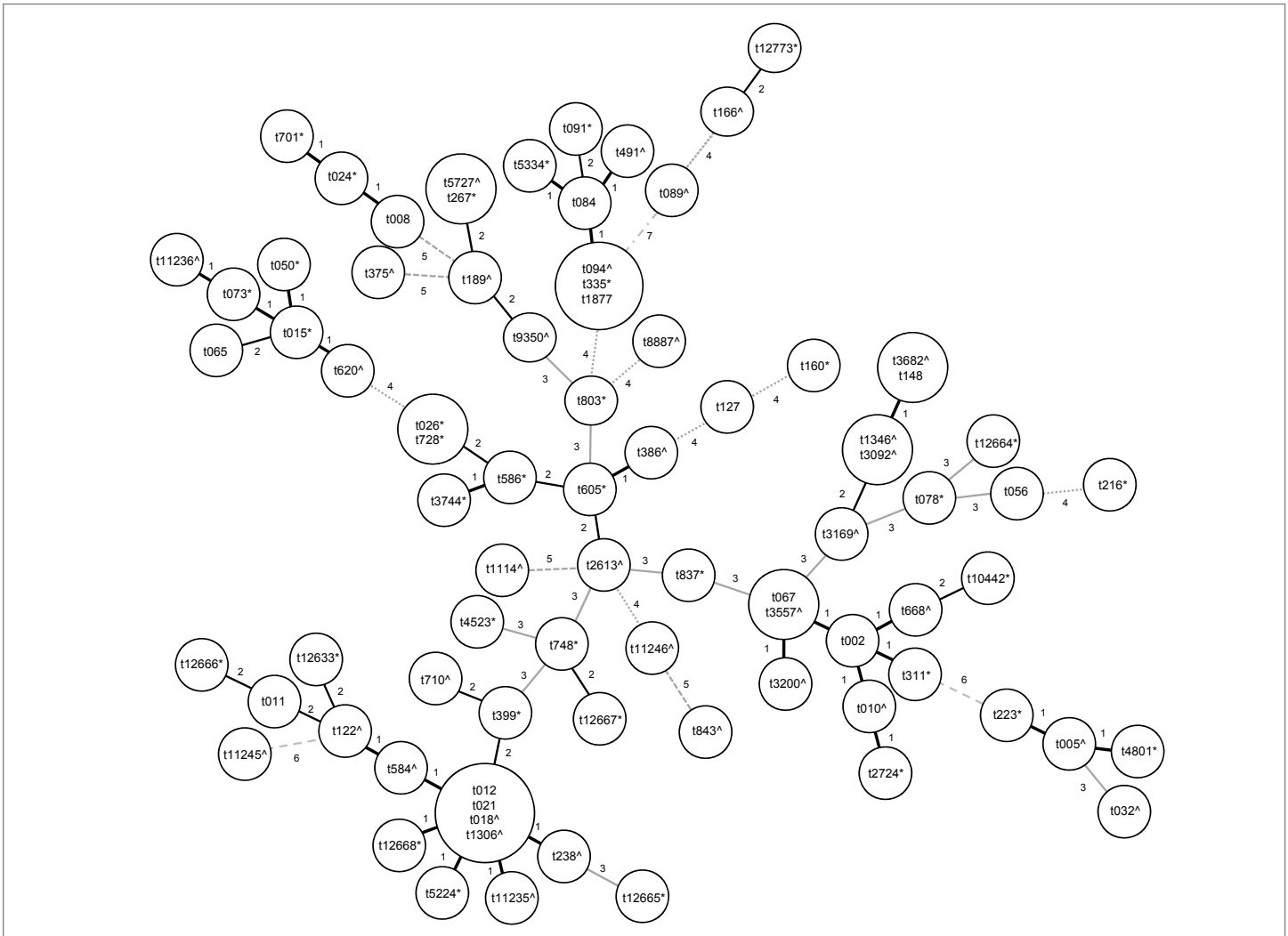

Figure 1: Clustering of spa types by minimal Spanning tree algorithm. Lines/numbers between circles represent the genetic distance between different spa-types. (*): spa types detected in river water; (^): spa types detected in urban effluents and ( ): shared spa types

Sample	Methicillin resistance	Sequence Type [ST]	*spa* type [number of isolates]
River water	MRSA	ST1	t127 [1]
		ST5	t10422 [1]
		ST106	t056 [1]
		ST125	t067 [28], t837 [1]
		ST228	t3744 [1]
		ST398	t011 [1]
	MSSA	ST5	t002 [13], t311 [1], t586 [1]
		ST6	t701 [1]
		ST7	t091 [1]
		ST8	t008 [3], t024 [2]
		ST12	t160 [1]
		ST15	t084 [5], t335 [1], t803 [2]
		ST22	t223 [1]
		ST25	t078 [6]
		ST26	t12664 [1]
		ST30	t012 [6], t399 [1], t5224 [1] t12633 [1], t12667 [1], t12668 [1]
		ST34	t12773 [1]
		ST45	t015 [2], t065 [1], t728 [2]
		ST59	t216 [1]
		ST72	t148 [3]
		ST97	t267 [4]
		ST101	t056 [1]
		ST125	t067 [1]
		ST398	t12666 [3]
		ST508	t050 [1]
		ST582	t605 [1]
		ST737	t4801 [1][a]
		ST707	t4523 [1]
		ST919	t12665 [1]
		ST2303	t1877 [1]
		ST2746	t073 [1]
		ST2747	t2724 [1]
		ST2748	t026 [1]
		ST2754	t5334 [1]
		ST2753	t748 [1]
Urban effluents		ST2812	t021 [1]
	MRSA	ST1	t127 [5], t386 [2]
		ST5	t002 [8], t668 [1]
		ST22	t032 [9]
		ST72	t148 [1], t9350 [1]
		ST125	t067 (69), t3557 [1]
		ST398	t011 [1]
		ST1094	t11245 [2]
		ST2674	t3200 [1]
		ST2676[b]	t843 [1][b]
	MSSA	ST5	t002 [3], t010 [2]
		ST8	t008 [1]
		ST9	t8887 [1]
		ST15	t084 [7], t094 [2], t491 [1], t1877 [1]
		ST22	t005 [1]
		ST25	t2613 [1]
		ST30	t012 [13], t018 [1], t021 [5], t122 [1], t238 [1], t584 [1], t710 [1], t1306 [1], t11235 [1]
		ST34	t089 [1], t166 [1]
		ST45	t065 [1], t620 [1], t11236 [1]
		ST49	t11246 [1]
		ST72	t1346 [4], t3092 [1], t3169 [1], t3682 [1]
		ST97	t5727 [1]
		ST106	t056 [3]
		ST121	t1114 [1]
		ST130	t843 [1]
		ST188	t189 [1]
		ST509	t375 [2]

Table 1: *Spa* types and sequence types [STs] of *S. aureus* found in river water and urban effluents.

MRSA: methicillin resistant *S. aureus*; MSSA: methicillin susceptible *S. aureus*; [a]PVL positive isolate; [b]*mec*C isolate [13]; bold letter: *spa* types and STs described in this study

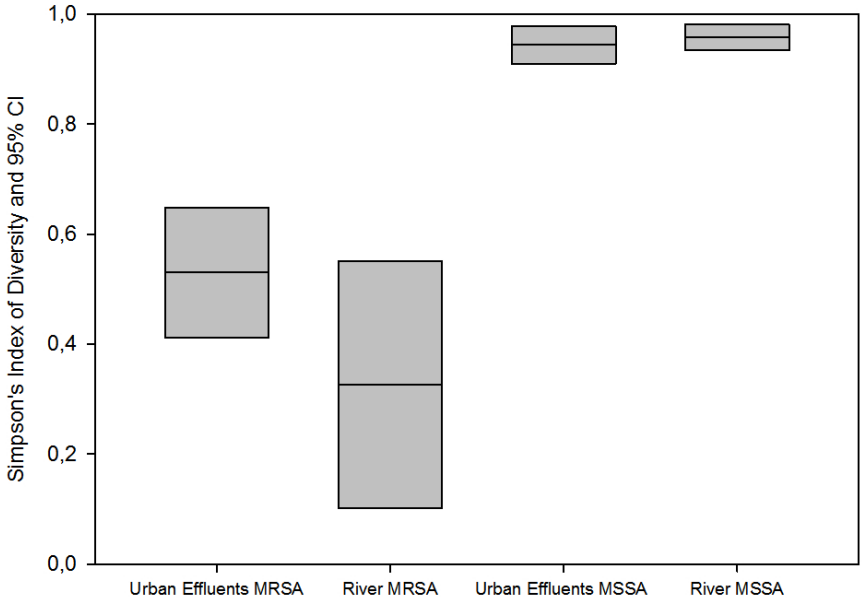

Figure 2: Genetic diversity of *S. aureus* isolates based on spa types: Simpson's Index of Diversity (Simpson's ID) and Jackknife pseudo-values confidence intervals (CI) at 95%. MRSA: methicillin resistant *S. aureus*; MSSA: methicillin susceptible *S. aureus*

(Table 1). Forty-two different *spa* types were detected in MSSA isolates from river water and 35 in urban effluents. On MRSA isolates, the number of different *spa* types detected from river water and urban effluent were 7 and 13, respectively (Table 1). This genetic diversity observed in the bacterial population of MSSA and MRSA isolates would reflect the *S. aureus* population in both water samples.

MSSA were genetically more diverse than MRSA isolates (Figure 2; $P<0.05$). Simpson's Index of Diversity (SID) based on *spa* types was 0.958 (95% CI: 0.934-0.981) for MSSA isolates from river water and 0.944 (95% CI: 0.910-0.978) for MSSA isolates from urban effluents (Figure 2; $P>0.05$). This genetic diversity observed in MSSA isolates is similar to that observed in previous studies [1]. Despite this high genetic diversity, some MSSA genotypes were more frequently isolated. Thus, the most frequent MSSA genotypes detected in river water were ST5/*spa* type t002 (13/81; 16.0%), ST30/*spa* type t012 (6/81; 7.4%), ST25/*spa* type t078 (6/81; 7.4%) and ST15/*spa* type t084 (5/81; 6.2%), while ST30/*spa* type t012 (13/67; 19.4%), ST15/*spa* type t084 (7/67; 10.4%) and ST30/*spa* type t021 (5/67; 7.5%) were the most frequent MSSA genotypes in urban effluents. Some of these frequent MSSA genotypes have been previously identified in human healthy carriers and patients [1,22,23].

Regarding MRSA isolates from river water and urban effluents, SID values were 0.326 (95% CI: 0.102-0.550)) and 0.530 (95% CI: 0.412-0.648) respectively ($P>0.05$).This low genetic diversity is due to the existence of predominant genotypes that included most of the MRSA isolates. In particular, the genotype ST125/*spa* type t067 represented the 82.4% (28/34), and the 67.6% (69/102) of the MRSA isolates from river water and urban effluents, in that order (Table 1). ST125-t067 has been geographically highlighted in *Spa*in representing the major MRSA genotype associated with nosocomial infections [1,24]. Other MRSA genotypes such as ST22-t032 and ST5-t002 (Table 1) have also been associated with human infections [4,22,24]. LA-MRSA were found in river water and in urban effluents, although typical genotypes such as ST398/*spa* t011 or its single locus variant ST1094 were only sporadically found in our study (Table 1).These results are likely due to the limited impact of animals in the areas of sampling, close to urban nucleus.

Our data demonstrated that the predominant MRSA and MSSA genetic lineages detected in urban effluent and river water were human associated genotypes. This is likely associated with the potential of colonized individuals to constantly release *S. aureus* into the environment [5,6,20], together with the capacity of *S. aureus* to persist in the water environments [6,10,25].

Conclusions

Our data emphasize the potential role of anthropogenic activities in the *S. aureus* dissemination throughout the water, and highlights the need to evaluate the circulation and persistence of this pathogen in the environment and its possible impact for public health.

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Conflicts of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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