Genotypic and Phenotypic Characterization of Staphylococcus aureus *in Children from Northern Portugal*

Schmid H., Lôpo N., Castro, A., Silva, J. and Teixeira, P.*

CBQF/Escola Superior de Biotecnologia – Universidade Católica Portuguesa, Porto, Portugal

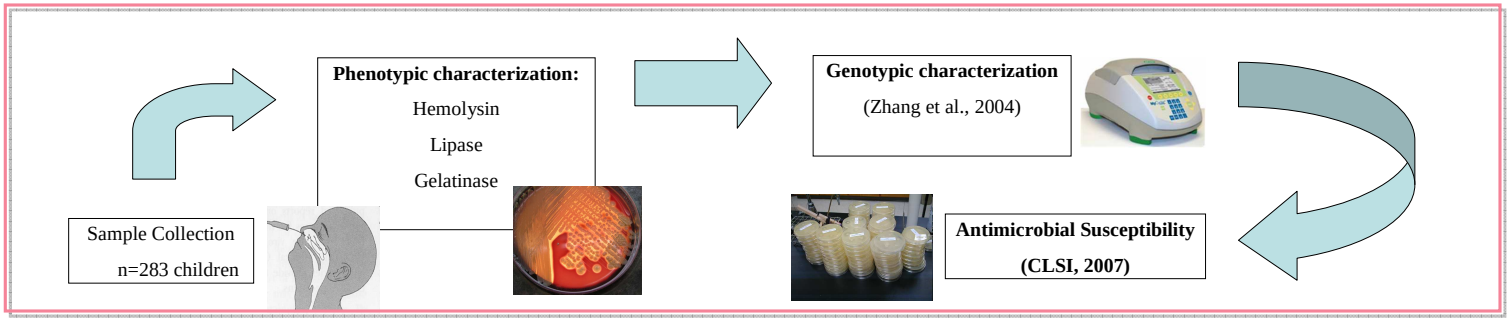
* **e-mail: pcteixeira@esb.ucp.pt**

CATÓLICA
UNIVERSIDADE CATÓLICA PORTUGUESA | PORTO
Escola Superior de Biotecnologia

INTRODUCTION

***Staphylococcus aureus* is well reported as an important agent of nosocomial and community-acquired infections in many European Countries (EFSA, 2009). *S. aureus* can be carried by healthy humans; up to 20% carry *S. aureus* in their nose, with no symptoms and are considered to be colonised. Infection can be moderate to severe and, in some cases fatal (Oguzkaya-Artan, 2008). Antibiotics are commonly used in prophylaxis and treatment of *S. aureus* infections. The percentage of infections caused by methicillin resistant *S. aureus* (MRSA) increased between 1997 and 2007 from 47.9 and 64.7%, respectively in intensive care units (ICU) in the United States (Burton et al., 2008). Methicillin resistance is mediated by an acquired penicillin binding protein, PBP2a, a peptidoglycan transpeptidase encoded by the *mec*A gene that has low affinity for beta-lactams. The purpose of this study was to characterize geno- and pheno-typically the populations of *S. aureus* in children from the North of Portugal, to determine to what extent isolates are resistant to the antibiotics and the proportions of MRSA in the Portuguese children population.**

MATERIALS & METHODS



RESULTS & DISCUSSION

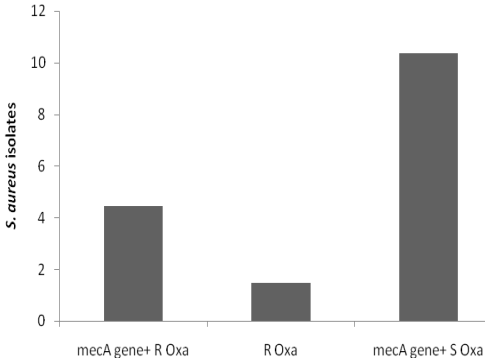


Fig. 1- Percentage of *S. aureus* isolates with and without the presence of *mecA* gene and with and without the resistance to oxacillin.

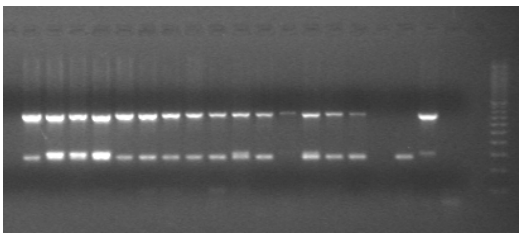


Fig. 2 – Detection of the 16S rRNA, *nuc* and *mec*A genes of *S. aureus* by Multiplex PCR assay. The represented bands correspond to a random selection of the isolates tested. Lane 1 - 15: selected strains; lane 17: Negative control (*S. epidermidis* DSM 20044); lane 18 Positive control (*S. aureus* ATCC 29213); lane 19: blank; lane 20: 1000-bp DNA ladder (BioRad).

One hundred and thirty five isolates (135/283; 47.7%) were confirmed to be *S. aureus* when the gene *nuc* and the target 16S rRNA were observed to be present simultaneously. The genotypic identification was totally in concordance with the results obtained for the phenotypic characterization namely, the Gram staining, fermentation of mannitol, Catalase and coagulase. Table 1 shows the analysis of the risk factors for *S. aureus* carriage.

Since many of the MRSA strains exhibit a hetero-resistance phenotype, detection of the *mec*A gene by molecular methods has become the reference method for confirmation of MRSA strains (Becker et al., 2006; Hososaka et al., 2007). In the present study, the *mec*A gene was detected in 15% of the overall *S. aureus* isolates. The results of the antibiotic sensitivity tests are summarized in Table 2.

Figure 1 illustrates the percentage of *S. aureus* isolates which were; i) positive for the presence of *mecA* gene and showing a MIC of oxacillin higher than 4 mg/l (4.4%); ii) negative for the presence of *mecA* gene and resistant to oxacillin (1.5%) and iii) positive for the presence of *mecA* gene and oxacillin-susceptible (10.4%). Hososaka et al., (2007) reported that from 480 *S. aureus* strains, 6 were MRSA but oxacillin-sensible (OS-MRSA).

Our results show that the carriage of MRSA exists among healthy children. Therefore continuing surveillance is needed to more accurately assess the prevalence, epidemiology of community-acquired infection and to develop strategies that will improve therapy and control the spread.

Table 2 - a antibiotic with no described value for the intermediate MIC; b S – Susceptible; c I – Intermediate; d R – Resistant, as defined by CLSI (2007).

	S[b]	I[c]	R[d]
Antibiotic	No. (%)	No. (%)	No. (%)
Ampicillin[a]	21 (15.6)	[a]	114 (84.4)
Chloramphenicol	132 (97.8)	--	3 (2.2)
Ciprofloxacin	116 (85.9)	19 (14.1)	--
Erythromycin	34 (25.2)	2 (1.5)	99 (73.3)
Gentamicin	133 (98.5)	--	2 (1.5)
Nitrofurantoin	109 (81)	--	26 (19)
Oxacillin[a]	126 (93.3)	[a]	9 (6.7)
Penicillin[a]	12 (8.9)	[a]	123 (91.1)
Rifampicin	132 (97.8)	--	3 (2.2)
Tetracycline	83 (61.5)	6 (4.4)	46 (34.1)
Vancomycin	135 (100)	--	--

REFERENCES

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- Oguzkaya-Artan M, Baykan Z, Artan C. (2008) Nasal Carriage of *Staphylococcus aureus* in Healthy Preschool Children. *Jpn J Infect Dis*, 61: 70-72

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