### **HIGH RATES OF COMMUNITY ACQUIRED**

### **PVL- MRSA INFECTIONS IN RABTA HOSPITAL OF TUNIS**

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## Introduction

- MRSA may be associated to healthcare and/or to the community infections
- Multiple MRSA clones have been identified in different countries and a number among them have been described as international or pandemic clones
- The major clone previously described is PVL positive community acquired MRSA
- This clone, which is spreading in Europe has an ST80, an agr3 allele predominately and a spa type t044

## **Objective**

# To Characterize in our hospital: the virulence factors Spa type Sequence type of MRSA strains in the community/hospital

## **Materiel and methods**

- Bacterial Strains (2005-2008): all MRSA strains isolated from bloodstream (25) were concerned and 34 other randomly strains were selected from different specimen
- Identification: S aureus was identified by standard microbiological methods
- Antimicrobial resistance was determined by the disk diffusion method in accordance with CA-SFM

## **Materiel and methods**

- Genomic DNA was extracted by using standard phenol-chloroform procedure
- The detection of gyr A gene, mec A gene, virulence factors genes and agr groups (1-4) were performed by multiplex PCR
- The SCC mec types (I-VI) was performed by amplifying regions within SCCmec, the ccr and the mec regions as described by Kondo and al.

## **Materiel and methods**

• MLST: performed as previously described by Enright et al., 2000.

The allelic profiles of MRSA strains were assigned on the basis of their MLST type using the MLST program (http://saureus.mlst.net/)

 Spa typing: Spa types were determined with the assistance of Ridom Staph Type software (Ridom GmbH, wurburg, Germany (Grisold et al., 2002)

Demographic and clinical Data

- 39 patients (66%) among the 59 studied had healthcare-associated infections (HAI)
- 20 patients (34%) had a community-acquired infection (CAI)

Age	HA-MRSA n=39	CA-MRSA n=20	Total n=59
Median age	41	36	41
Ranging years	4 days -75 years	11 - 72 years	4 days-75 years

### **Specimen origins**

Specimens	HA-MRSA n=39	CA-MRSA n=20	Total n=59
Pus	10	14 (70%)	24
Blood	<b>25 (64%)</b>	0	25
Pleural fluids	2	0	2
Pus of nose	0	4	4
Armpits	1	1	2
Catheters	2	0	2

70% of CA-MRSA strains were isolated from pus

• Blood (64%) was the major source of HA-MRSA strains

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### **Clinical units**

	HA-MRSA n=39	CA-MRSA n=20	Total n=59
Intensive care Dermatology Neonatology Cardiology Otolaryngology Orthopaedy Haematology Surgery Infectious	12 (31%) 10 (26%) 6 3 0 2 1 3 2	0 <b>17 (85%)</b> 0 0 2 0 1 0 0	12 27 6 3 2 2 2 2 3 2
diseases	2	U	۷

85% of patients with CA-MRSA were seen by Dermatology department

HA-MRSA were isolated in the intensive unit care in 31% and Dermatology department in 26% of cases

#### Distribution of isolates according agr group and toxin genes

	Clones	agr	Toxin gene	SCC mec	ST	Number
(	ST 80 ST 80	3 3	edin, PVL edin, PVL, sed, ser	IV IV	80 80	26 1 ]27
	Paediatric Paediatric Paediatric	2 2 2	sem, seo, Edin sem, seo sem, seo, Edin, tst	IV IV IV IV	5 5 5	4 7 4 15 4
	Iberian		Sea	IV	247>	14
	Hungarian	1	sea, sec, sel	III	241	1
	Not attached	1	sea, sel K, sel Q	CCr A1,	241	1
	to any clone	3	sea, seh	CCRA2, Classe B	1	1

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### **Characteristics of ST 80 clone**

- The ST80 clone was the most frequently isolated: 27/59 (47%)
- PVL positive isolates caused 14 (70%) of the community acquired infection and 14 (36%) of the hospital acquired infection
- Three deaths occured
- 24 of the 27 isolates with PVL gene toxin were resistant to tetracyclin, 23 to kanamycin, 22 to fusidic acid and 17 to erythromycin
- All isolates were sensitive to gentamycin

### **Characteristics of paediatric pandemic clone (25%)**

- 11 of the 15 patients with egc toxin positives MRSA were HA and 4 were in the community
- Four deaths occurred
- All strains were resistant to penicillin, kanamycin and tetracyclin. 8 were resistant to tobramycin, 7 to erythromycin and 6 to fusidic acid
- Only one isolate was resistant to gentamycin

### **Characteristics of Iberian clone (23%)**

- 13 of the14 patients with Iberian clone had acquired hospital infection
- 8 deaths occurred (57%)
- All isolates were resistant to kanamycin and tobramycin. 12 isolates were resistant to ofloxacin and gentamycin, 10 to rifampicin, 3 to fosfomycin and fusidic acid

 PVL positive CA-MRSA emerged 1<sup>st</sup> in North America (Naimi et al., 2001) and Australia (Turnidge et al., 2000), then they have spread in Europe (Dufour et al., 2002) and were recently detected in north Africa (B Nejma et al., 2006; Ramdani-Bouguessa., 2006) and currently in Tunisia

These data suggest that ST80 clone is an epidemiological clone that has rapidly spread all over the world

- In our study, 63% of PVL MRSA were isolated from discharge and 37% from septicemia. In fact PVL is a toxin associated with S aureus strains causing severe skin infections (Dufour et al., 2002)
- The ST 80 clone was first described as CA-MRSA clone but in our study it was present in both HA-MRSA and CA-MRSA (Vandenesh., 2003)
- PVL caused the 70% of community-acquired infections and 36% of hospital acquired infections and this finding is similar to result of Ramdani and al. in Algeria (2006)

- Vandenesh and al showed that PVL positive ST 80 CA-MRSA strains are usually resistant to oxacillin, kanamycin, tetracyclin and fusidic acid (Marquet et al., 2004) and it's particularly worrisome that we detected multidrug resistant
- As well as our study which CA-MRSA ST 80 showed resistance to fusidic acid, tetracyclin and kanamycin, similar to CA-MRSA isolated in Algeria (Ramdani-Bouguessa., 2006)
- In contrast other studies conducted in Tunisia (B Nejma et al., 2006) and Egypt (Enany et al., 2009) showed that isolates were susceptible to tetracyclin and fusidic acid

- The ST5 SCCmec IV Pediatric clone is one of the major hospital acquired MRSA clones widely distributed worldwide (Vivoni et al., 2006; Tenover et al., 2006; Sa-Leao et al., 1999)
- It corresponded to both TSS and suppurative infections usually resistant to oxacillin, kanamycin and tobramycin and intermediary to fusidic acid (Aires de Sousa et al., 2005)
- In our study all isolates were resistant to kanamycin but only, 8 to tobramycin and 6 to fusidic acid

- The Iberian clone is one of the pandemic clones and had been identified in several countries such as Spain and Portugal (Argudin et al., 2009)
- In our study MRSA Iberian clone strains were multi-résistant to antibiotics
- This multiresitsance could be explained by the presence of several resistance genes integrated in different locations on the chromosome than the mec cassette (Ito et al., 2004)

### **Conclusions**

The combination of molecular typing techniques, allowed us to

- demonstrate the High prevalence of ST 80 clone (46%)
- This clone was described by B Nejma and al in Monastir hospital (Tunisia) but Iberian and pediatric clones were described for the first time in our study