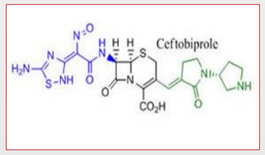
New drugs active against both rifampin- and methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from an Italian surveillance study

Campanile Floriana, Bongiorno Dafne, Benvenuto Sabrina, Cafiso Viviana, Santagati Maria and Stefani Stefania
Department of Biomedical and Biotechnological Sciences (BIOMETEC) - MMAR*Lab* - University of Catania, Italy

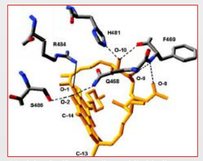
P1344

The aims of this study were: i) to evaluate the *in vitro* antimicrobial activity of ceftobiprole and dalbavancin, against RIF-R MDR-MRSA isolates from an Italian surveillance study, and ii) to trace their relationship with hVISA and DNS phenotypes; iii) to analyze mutations in *rpo*B gene, correlated with rifampicin-resistance.



Ceftobiprole medocaril is a newly approved drug in Europe for the treatment of hospital-acquired pneumonia (HAP) and community-acquired pneumonia (CAP) in adults. anti-MRSA ceftobiprole represents a remarkable evolution of the cephalosporin class, useful for the therapeutic *armamentarium* of staphylococcal infections (1, 2).

Dalbavancin is a lipoglycopeptide antibiotic recently approved for acute bacterial skin and skin structure infections (ABSSSIs) (3, 4). Recent studies demonstrated that dalbavancin exerts an *in vitro* activity 4-8 times more potent than vancomycin against MRSA, but only limited data have described its activity against MDR-MRSA, including hVISA and VISA (5, 6).



Model of rifampin (in gold) binding to important residues in the of *S.aureus* wild type β-sub unit (adapted by O'Neill AJ et al. AAC 2006; 50:298-309).

Multiresistance (MDR) represents the major problem among MRSA isolates in hospital settings. Rifampin is one the major anti-MRSA drugs, usually used in a combination therapy for the treatment of severe infections. The frequency of rifampin-resistant (RIF-R) *Staphylococcus aureus* isolates have rapidly increased. In Italy, MRSA isolates showed a higher percentage of RIF-R (16.4%) in 2012, with respect to the European trend (5.7%), as reported by the annual ECDC surveillance report (7, 8).
In *S.aureus*, the main mutations responsible for rifampicin resistance are clustered in an extremely conserved region of the RNA polymerase beta-subunit, called "rifampicin resistance-determining region" (RRDR), spanning from amino acid (aa) 463 to 550. In particular, in this sequence, two clusters are closely associated with rifampicin resistance: Cluster I (aa 462-488) and Cluster II (aa 515-530) (9).

Table 1. The RIF-R MDR-MRSA molecular characteristics and the MIC distributions of the new drugs in study

Clone (strain n.)	hVISA %	DNS %	*rpo*B mutations (strain n.)	Ceftobiprole distribution								Dalbavancin distribution						
				Range (mg/L)	0.25	0.5	1	2	4	MIC_{50}	MIC_{90}	Range (mg/L)	0.06	0.125	0.25	0.5	MIC_{50}	MIC_{90}
ST228/SCC*mec* I (22)	86.3	18.2	H481N (13); H481N+I527M (4); H481D (3); H481N+S529L (1); S464P (1)	1-4	0	0	4	9	9	2	4	0.125-0.5	0	3	11	8	0.25	0.5
ST5/SCC*mec* II (7)	28.5	60	H477D (3); H481N (2); H481D (1); H481N+S529L (1)	0.25-4	1	1	1	2	2	2	4	0.06-0.5	1	4	1	1	0.125	0.25
ST239-241/SCC*mec* III (8)	37.5	12.5	H481N (4); H481N+L466S (2); H481N+S529L (1); H481N+A477T (1)	2-4	0	0	0	6	2	2	4	0.06-0.25	1	6	1	0	0.125	0.125
ST8/SCC*mec* IV (9)	55.5	22.5	H481N (5); R484H+D471G (2); H509R (1); A477D(1)	0.25-2	1	5	2	1	0	0.5	1	0.125-0.25	0	3	6	0	0.25	0.25
ST22/SCC*mec* IVh (2)	50	50	H481N (1); H481Y (1)	0.5-1	0	1	1	0	0	1	1	0.125	0	2	0	0	0.25	0.25
Sporadic clones (2)	0	0	S486L (1); H481N+S529L (1)	0.25-2	1	0	0	1	0	0.25	2	0.125-0.25	0	1	1	0	0.125	0.25

Figure 1: Distribution of the different *rpo*B mutations

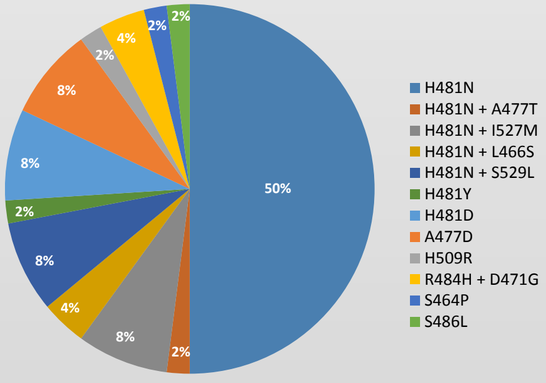
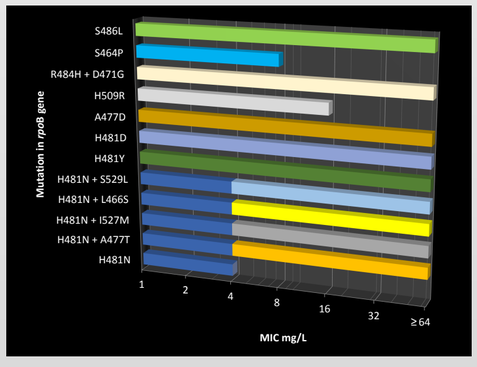


Figure 2: Correlation between rifampicin-resistance and *rpo*B mutations



The RIF-R isolates analyzed in this study, with a high percentage of hVISA, represent the major MDR-MRSA clones circulating in Italy. The hVISA phenotype was found in 60% of all RIF-R strains, and 11 isolates were daptomycin non-susceptible (DNS).
Among RIF-R MDR-MRSA strains, five main clones were found: ST228/SCC*mec* I (*spa* type t041-t001-t1159-t3217-t4072); ST5/SCC*mec* II (*spa* type t2154-t3217-t002); ST239-241-SCC*mec* III (*spa* type t030-t037); ST8/SCC*mec* IVc (*spa* type t008-t2450-t121); ST22/SCC*mec* IVh (*spa* type t020-t902); sporadic clones. The most common clonal type observed among these strains was associated with the spread of ST228-SCC*mec* I, among which 86.3% were hVISA, in which 5 different *rpo*B mutations were found (Tab. 1).
All the amino acid substitutions we identified were present in the RRDR, almost all in Cluster I, with the exception of I527M an S529L, falling into Cluster II. Only the amino acid substitution H509R was in a region between cluster I and II, conferring moderate value of RIF resistance (Fig. 1 and 2).

Ceftobiprole inhibited 74% of RIF-R MDR-MRSA isolates (MICs ≤2mg/L). **No isolates showed MIC values higher than 4 mg/L.**
ST228, ST5 and ST239 had ceftobiprole MIC_{50} values of 2 mg/L, while those isolates with ST8 and ST22 had MIC_{50} values of 0.5 and 1 mg/L, respectively.
Only 13 MRSA strains showed resistance to ceftobiprole (MIC value 4 mg/L), not associated with a specific *rpo*B mutation. 54% of these strains showed the hVISA phenotype (7/13), all belonging to ST228-SCC*mec* I. Only 2 DNS strains showed ceftobiprole resistance (Tab. 1).

Dalbavancin inhibited 42% of RIF-R MDR-MRSA isolates (MICs ≤0.125mg/L). No isolates showed MIC values higher than 0.5 mg/L.
ST5 and ST239, and sporadic clones had dalbavancin MIC_{50} values of 0.125 mg/L.
20 strains showed a MIC value of 0.25 mg/L, only one doubling dilution above the susceptibility breakpoint, and only 9 strains showed a higher MIC value of 0.5 mg/L. 65% of these strains showed the hVISA phenotype (19/29), manly belonging to ST228-SCC*mec* I (14/19).
5 DNS showed dalbavancin resistance (Tab. 1).

In this study, we identified 13 diverse amino acid substitutions. H481N mutation was the most prevalent one. The majority (n. 25, 50%) of the 50 RIF-R MDR-MRSA isolates harbored the amino acid substitution of an histidine with an asparagine, at 481 locus (H481N). Two novel *rpo*B variants never described before, were identified (H509R; D471G) (Fig. 1)

Low-level resistance to rifampicin is attributed to H481N. Different mutations at the equivalent locus: H481D and H481Y (found in a DNS/hVISA strain) showed high-level resistance. High-level resistance is also ascribed to multiple mutations, including H481N, thereby indicating a step-by-step mechanism in resistance development (probably due to the exposition to rifampicin-therapy) (Fig. 2).

Ceftobiprole and dalbavancin demonstrated a wide spectrum of *in vitro* activity, with variation in MIC values according to clonal types, and with an excellent *in vitro* activity against RIF-R MDR-MRSA clinical isolates, especially with regards to hVISA and DNS phenotypes. Dalbavancin had potent activity against staphylococcal isolates with vancomycin MICs⩾1.0 mg/L, and also exhibited more potent *in vitro* activity than vancomycin and daptomycin. The different *rpo*B mutations did not altered the MIC values of ceftobiprole and dalbavancin.

References
1. Scheeren TW. Future Microbiol. 2015; 10:1913-28. **2.** Holmes NE and Howden BP. CurrOpin Infect Dis. 2014; 27:471-8. **3.** Klinker KP. Et al. ClinTher. 2015 Dec 1;37:2619-36. **4.** Jones RN., et al. DMID 2013; 75: 304-307. **5.** McCurdy SP, et al. AAC 2015; 59:5007-9. **6.** Smith JR, et al. Infect Dis Ther 2015;4:245-58. **7.** Campanile F. et al., JGAR 2015;3:247-254. **8.** EARS-Net, http://ecdc.europa.eu. **9.** Wichelhauser TA al., AAC 1999; 43: 2813–2816. **10.** O'Neill AJ et al. AAC 2006; 50:298-309