Comparative Study of the Phagocytosis and Intracellular Susceptibility to Antibiotics Of a Pig-related ST-398 Methicillin-Resistant *S. aureus* (A-MRSA) vs Hospital-Acquired MRSA in a Model of THP-1 Macrophages

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Abstract

Background
MRSA is a global scourge worlwide in the hospital and community settings, with limited treatment options, and frequent relapses (or persistence of infection) related to intracellular survival (Garzoni and Kelley, Trends Microbiol. 2009, 17:59-65). In the last years, additional reservoirs of MRSA have been identified in farm animals, and particularly in pigs (Huber et al, Euro Surveill. 2010, 22;15(16)). Since these strains have the potential for cross-transmission between animals and humans, we have examined the phagocytosis and intracellular susceptibility to antibiotics of a recent belgian A-MRSA ST-398 of porcine origin isolated from a pig-handler.

Methods
Strains ATCC 33591 (HA-MRSA) and N7112046 (A-MRSA ST-398) were used thorough. MICs were determined by microdilution method in Mueller Hinton Broth (pH 7.4). Intracellular survival and antibiotic activities were measured on bacteria phagocytized by THP-1 macrophages (ATCC TIB-202), a human myelomonocytic cell line displaying macrophage-like activity. Bacterial phagocytosis was studied in THP-1 cells exposed to 4 bacteria/cell for 1 h (Olivier et al, J. Infectious Diseases 2009, 200:1367-1370). Intracellular activity of antibiotics was determined after 24 hours exposure to a drug concentration corresponding to the Cmax of the corresponding antibiotic (control cells: gentamicin [0.5 x MIC] to prevent extracellular growth; Barcia-Macay et al. Antimicrob. Agents Chemother., 2006, 50 :841-851]).

Results.
Within THP-1 macrophages, both strains were internalized to a similar extent (~ 10^6 CFU/mg of cell protein) and grew at a similar rate over 24 h (up to 10^7 to $5x10^7$ CFU/mg of cell protein). MICs (mg/L) for HA-MRSA vs. A-MRSA) were: vancomycin and linezolid, 1 and 1; rifampicin, 0.06 and 0.01; moxifloxacin, 0.125 and 0.06; daptomycin, 0.125 and 0.5; quinupristin/dalfopristin [w/w, 30/70], 0.5 and 0.25. Intracellular activities at Cmax (decrease log CFU/mg prot. over 24 h from post-phagocytosis inoculum) were for HA-MRSA vs. A-MRSA: vancomycin (50 mg/L), -0.6 ± 0.1 *vs.* –0.4 ± 0.1; linezolid (20 mg/L), -0.7 ± 0.1 *vs.* –0.5 ± 0.1; rifampicin (4 mg/L), -1.5 ± 0.1 *vs* -1.5 ± 0.1; moxifloxacin (4 mg/L), -1.4 ± 0.1 *vs.* –1.5 ± 0.1 ; daptomycin (77 mg/L), -1.5 ± 0.1 *vs* -1.5 ± 0.1; quinupristin-dalfopristin (10 mg/L), -1.5 ± 0.1 *vs.* –2.1 ± 0.1).

Conclusions
A-MRSA ST-398 appears to invade and to multiply in THP-1 macrophages alike HA-MRSA, and shows a similar susceptibility to antibiotics. Of note, this activity remains modest, especially for vancomycin and linezolid, suggesting that A-MRSA may, as other MRSA, cause easily persistent and relapsing infections even in patients treated with these antibiotics.

Background and aim

Treatment failures and relapses are frequently observed with *Staphylococcus aureus* infections. This may be ascribed to multi-drug resistance (among which Methicillin-Resistance in *S. aureus*) and to the capacity of this organism to survive within eukaryotic cells (Sinha et al, *Int J Med Microbiol.* 2010, 300:170-5).

Over the past decades, MRSA is becoming an increasingly problematic organism. While long confined to the hospital setting, this phenotype is now emerging in the community as well as in pets, farm animals, and their human contacts (see for review: Catry *et al*, *Epidemiol Infect.* 2010, 138:626-44). Screening of pig and pig farmers revealed high prevalence of MRSA sequence type (ST) 398 (Witte *et al*, *Emerg Infect Dis.* 2007, 13:255-8; van Belkum *et al, Emerg Infect Dis.* 2008, 14:479-83), an unusual phenotype in humans.

Since these MRSA isolates have the potential for cross-transmission between animals and humans, we have examined the ability of a recent Belgian ST-398 animal MRSA (A-MRSA) of porcine origin (and isolated from a pig-handler) to be internalized by THP-1 macrophages, as well as the activity of antibiotics towards intraphagocytic bacteria.

Methods

• Bacterial strains. MRSA strain ATCC 33591 and N7112046 (A-MRSA ST 398) were used in this study.

• Susceptibility testings. MICs were determined in Mueller Hinton Broth (pH 7.4) by the microdilution method.

• Cells, cell infection and assessment of antibiotic activity. THP-1 macrophages (ATCC TIB-202; American Tissue Culture Collection, Manassas, VA), a human myelomonocytic cell line displaying macrophage-like activity, were used. Bacterial phagocytosis (1 h incubation) was studied using a multiplicity of infection of 4 bacteria per cell (Olivier et al, *J infect Dis.* 2009, 200:1367-1370). Intracellular activity of antibiotics was determined after 24 h exposure to a fixed extracellular drug concentration (corresponding to their respective human Cmax). Control cells were incubated in the presence of gentamicin (0.5 x MIC) to prevent extracellular growth (Barcia-Macay *et al, Antimicrob. Agents Chemother.* 2006, 50:841-851).

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Results

Susceptibility testings

MICs (mg/L)	HA-MRSA (ATCC 33591)	A-MRSA (ST398)
Vancomycin	1	1
Linezolid	1	1
Rifampicin	0.06	0.01
Moxifloxacin	0.125	0.06
Daptomycin	0.125	0.5
Quinupristin/Dalfopristin (w/w, 3:7)	0.5	0.25

***HA-MRSA (ATCC 33591)* and ST-398 *Animal MRSA* show similar susceptibility to antibiotics**

Bacterial internalization within THP-1 cells

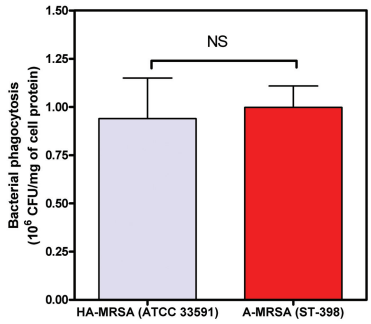

Figure 1. Comparative phagocytosis of MRSA (HA-MRSA vs A-MRSA) within THP-1 macrophages (1 h incubation) using a multiplicity of infection (MOI) of 4 bacteria per cell. Results are means ± SD of three independent determinations.

***HA-MRSA (ATCC 33591)* and ST-398 *A-MRSA* are as efficently internalized by THP-1 macrophages**

Comparative intracellular susceptibility to antibiotics

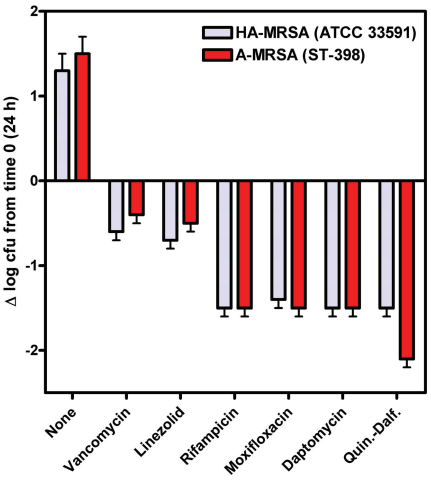

Figure 2. Comparative activity of antibiotics towards intraphagocytic HA-MRSA (ATCC 33591) and A-MRSA (ST-398). Intracellular activity was determined using a fixed extracellular concentration corresponding to the Cmax reported for humans in the litterature (vancomycin, 50 mg/L; linezolid, 20 mg/L; rifampicin, 4 mg/L; moxifloxacin, 4 mg/L; daptomycin, 77 mg/L; quinupristin-dalfopristin, 10 mg/L). The ordinate shows the change in cfu (log scale) per mg of cell protein observed after 24 h incubation, in comparison with the original, post-phagocytosis inoculum. Results are means ± SD of three independent determinations.

***HA-MRSA (ATCC 33591)* and ST-398 *Animal MRSA* show similar growth and susceptibility to antibiotics intracellularly**

Conclusions

- **The Animal MRSA ST-398 used in the present study appears to efficiently invade and multiply within THP-1 macrophages, and shows a similar susceptibility to antibiotics as a HA-MRSA strain.**
- **However, the intracellular activity of antibiotics remains modest (especially for vancomycin and linezolid that are the current therapeutic options for MRSA infections in Belgium), which may contribute to persistence and relapses in patients treated with these antibiotics.**

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