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 worldwide in the hospital and community settings, with limited treatment options, and frequent relapses (or persistence of infection) related to intracellular survival (Gedlott and Kelley, Trends Microbiol. 2006, 17:58-65). In the last years, additional reservoirs of MRSA have been identified in farm animals, and particularly, in pigs (Huber et al., Emerg. Infect Dis. 2008, 14:1753-1758). 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

Sinha (ATCC 35395) and N7112046 (A-MRSA ST-398) were used throughout. MICs were determined by microdilution method in Mueller Hinton Broth (pH 7.4). Intracellular survival and antibiotic activities were measured on bacteria phagocytophagized 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 (Oliver et al., J. Clin. Microbiol. 2009, 47:3399-3403). Intracellular activity of antibiotics was determined after 24 hours exposure to a drug concentration corresponding to the Cmax reported for humans in the literature (vancomycin, 50 mg/L; linezolid, 20 mg/L; rifampicin, 4 mg/L; moxifloxacin, 4 mg/L; daptomycin, 2 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.

Results

Comparative intracellular susceptibility to antibiotics

Figure 2. Comparative activity of antibiotics towards intraphagocytic HA-MRSA (ATCC 35395) and A-MRSA (ST-398). Intracellular activity was determined using a fixed extracellular concentration corresponding to the Cmax reported for humans in the literature (vancomycin, 50 mg/L; linezolid, 20 mg/L; rifampicin, 4 mg/L; moxifloxacin, 4 mg/L; daptomycin, 2 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.

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.

Conclusions

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, 300:170-9).

Over the past decades, MRSA is becoming an increasingly problematic pathogen. 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 MRSA.

Methods

• Bacterial strains. MRSA strain ATCC 35395 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 (Oliver et al., J. Infectious Diseases 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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