Disponible en ligne sur ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com

PATHOLOGIE BIOLOGIE

Pathologie Biologie 59 (2011) 199–206

Original article

Prevalence of resistance phenotypes and genotypes to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in Tunisian Bone Marrow Transplant Center

Prévalence des génotypes et des phénotypes de résistance aux macrolides, lincosamides et streptogramines chez les cocci Gram positifs isolés au centre national de greffe de moelle osseuse de Tunis

O. Bouchami [a,b,*], W. Achour [a,b], A. Ben Hassen [a,c]

[a] *Laboratoire du centre national de greffe de moelle osseuse, 1006 Tunis, Tunisia*
[b] *Laboratory service, centre national de greffe de moelle osseuse, Tunisia*
[c] *Laboratoires service, faculté de médecine, centre national de greffe de moelle osseuse, Tunisia*

Received 11 October 2008; accepted 18 March 2009
Available online 28 May 2009

Abstract

To investigate the prevalence of resistance to macrolide, lincosamide and streptogramin (MLS) antibiotics in Gram-positive cocci isolated in a Bone Marrow Transplant Center of Tunisia, we tested the antibiotic susceptibility of 172 clinical isolates of *Staphylococcus epidermidis, Streptococcus mitis* and *Enterococcus faecium* to macrolide erythromycin and spiramycin, the lincosamide clindamycin and the streptogramin pristinamycin. These three groups of organisms were mostly resistant to macrolides and lincosamide, but were commonly susceptible to pristinamycin. The resistance phenotypes of erythromycin-resistant isolates were determined by the five-disc test with erythromycin, spiramycin, lincomycin, clindamycin and pristinamycin, which showed that most exhibited constitutive MLS resistance. In order to determine the prevalence of the resistance genotypes and the resistance mechanisms, the prevalence of the erythromycin resistance methylase (*erm*) (A), *erm*(B), *erm*(C), *msr*(A) and macrolide efflux (*mef*) (A) genes in the erythromycin-resistant isolates was identified by polymerase chain reaction (PCR) analysis. The resistance was due mainly to the presence of *erm*B in *E. faecium* (80%), *erm*C in *S. epidermidis* (53%) and *mef*A in *S. mitis* (65%).

Keywords: MLS; Erythromycin; *Staphylococcus epidermidis*; *Enterococcus faecium*; *Streptococcus mitis*

Résumé

Pour étudier la prévalence de la résistance aux macrolides, lincosamides et streptogramines (MLS) chez les cocci Gram positifs isolés dans le Centre national greffe de moelle osseuse de Tunis, nous avons testé la sensibilité à l'érythromycine et la spiramycine (macrolides), à la clindamycine (lincosamide) et à la pristinamycine (streptogramine) chez 172 isolats cliniques de *Staphylococcus epidermidis, Streptococcus mitis* et *Enterococcus faecium*. Ces trois groupes d'organisme sont pour la plupart résistants aux macrolides et au lincosamide, mais sont généralement sensibles à la pristinamycine. Les phénotypes de résistance des souches résistantes à l'érythromycine ont été déterminés par les cinq disques d'antibiotiques suivants : l'érythromycine, la spiramycine, la lincomycine, la clindamycine et la pristinamycine et ont montré que la plupart présentaient une résistance constitutive aux MLS. Afin de déterminer la prévalence des génotypes de résistance aux MLS ainsi que les mécanismes de cette résistance, la prévalence des gènes de résistance chez les souches résistantes à l'érythromycine, *erythromycin resistance methylase* (*erm*) (A), *erm*(B), *erm*(C), *msr*(A) et *macrolide efflux* (*mef*) (A) a été

* Corresponding author. National Bone Marrow Transplant Center, Street Djebel Lakhdhar, Bab Saadoun, 1006 Tunis, Tunisia.
E-mail addresses: onsboucham@yahoo.fr (O. Bouchami), wafaachour@gmail.com (W. Achour), assiabenhassen@yahoo.fr (A. Ben Hassen).

0369-8114/$ – see front matter
doi:10.1016/j.patbio.2009.03.010

déterminé par *polymerase chain reaction* (PCR). La résistance est principalement due à la présence des gènes, *ermB* chez *E. faecium* (80 %), *ermC* chez *S. epidermidis* (53 %) et *mefA* chez *S. mitis* (65 %).

Mots clés : MLS ; Érythromycine ; *Staphylococcus epidermidis* ; *Enterococcus faecium* ; *Streptococcus mitis*

1. Introduction

The macrolide, lincosamide and streptogramin (MLS) antibiotics are chemically distinct, but have similar inhibitory effects on bacterial protein synthesis. They are widely used in the treatment of Gram-positive infections. The expanded use of these antibiotics has been accompanied by increased numbers of resistant strains among these bacteria [1,2]. Resistance to MLS in Gram-positive cocci is mediated by a methylase encoded by erythromycin resistance methylase (*erm*) genes [3]. Methylases cause a conformational change in the prokaryotic ribosome, leading to reduce binding of MLS antibiotics to target site in the 50 S ribosomal subunit. The phenotypic expression of MLS resistance can be either inducible or constitutive [4,5]. Another mechanism of inducible resistance to erythromycin is conferred by the gene *msr*A, which encodes an ATP-dependent efflux pump have been found in staphylococci. A new mechanism of resistance to macrolides, based on an efflux system, has recently emerged among *Streptococcus pyogenes* and *Streptococcus pneumoniae*. It is due to the presence of macrolide efflux (*mef*) genes conferring the M phenotype, which characterized by resistance to 14 and 15-membred macrolides and susceptibility to lincosamide and streptogramin antibiotics [6]. Genes of the *mef* class have also been found in other Gram-positive genera, including *Coynebacterium, Enterococcus, Micrococcus* and *Streptococcus* [7].

In order to investigate the prevalence of MLS resistance in the Tunisian Bone Marrow Transplant Centre, we compared the in vitro activities of several MLS antibiotics against 172 clinical isolates of *Staphylococcus epidermidis, Streptococcus mitis* and *Enterococcus faecium*. In addition, the resistance phenotype was determined by the five-disc test, using erythromycin, spiramycin, lincomycin, clindamycin and pristinamycin. The resistance genotype was determined by polymerase chain reaction (PCR) analysis of the erythromycin resistant isolates for the presence of five representative MLS resistance genes, *erm*(A), *erm*(B), *erm*(C), *msr*(A) and *mef*(A).

2. Material and methods

2.1. Bacterial strains

A total of 172 clinical isolates of Gram-positive cocci, comprising 39 methicillin-resistant *S. epidermidis* (MRSE), 38 methicillin-susceptible *S. epidermidis* (MSSE), 50 *S. mitis* and 45 *E. faecium*, were collected from 83 neutropenic patients hospitalised at different units of the Bone Marrow Transplant Centre of Tunisia in 2002. The strains were recovered from various clinical specimens. *S. epidermidis* strains were isolated from blood cultures ($N = 55$) and central venous catheters ($N = 22$); *E. faecium* isolates were collected especially from stool cultures ($N = 40$), respiratory tract ($N = 2$) and different sites ($N = 3$); *S. mitis* strains especially from systematic nasopharyngeal specimens ($N = 42$), upper respiratory tract ($N = 5$) and other sources ($N = 3$). Multiple isolates from the same patient were avoided.

These isolates were identified to the species or genus level by means of conventional methods as previously described as well as by using the following commercial identification systems: the Api 20 strep system for the identification of enterococci and streptococci (B. M., La-Balmales-Grottes, France) and Api ID 32 staph system (for staphylococci identification) (BioMérieux, Marcy-l'Étoile, France).

2.2. Reference strains

The reference strains used in the present study were: *S. epidermidis* Who 23, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *E. faecalis* V583, *E. faecium* BM4147 and *S. pneumonia* ATCC 49619 (MICs quality control) [8,9]. *S. pneumonia* HM28 (*erm*B), *S. pneumonia* O2J1175 (*mef*A), *S. aureus* HM1051 (*erm*A + *erm*C), *S. aureus* HM1054/R (*erm*C), *S. aureus* S5 (*msr*A) (PCR quality control) [10].

2.3. Antibiotic susceptibility testing

The pattern of resistance to antimicrobial agents including erythromycin and spiramycin (a 16-membred macrolide), lincomycin and pristinamycin (an oral streptogramin) was determined by the disc diffusion method using Muller Hinton (MH) agar (for staphylococci) and MH medium (bioMérieux) supplemented with 5% horse blood and incubated at 37 °C for 24 h in an aerobic atmosphere under 5% CO_2 (for streptococci and enterococci) as recommended by the Comité de l'antibiogramme de la Société française de microbiologie (CAS-FM) [11]. Commercially available discs loaded with the following antibiotics were used:

- penicillin G (6 µg);
- oxacillin (5 µg);
- amoxicillin (25 µg);
- amoxicillin–clavulanic-acid (20/10 µg);
- cefalotin (30 µg);
- cefotaxim (30 µg);
- imipenem (10 µg);
- streptomycin (10 UI);
- streptomycin (500 µg);
- kanamycin (30 µg);
- kanamycin (1000 µg);
- gentamicin (15 µg);