New Gene Responsible for Resistance of Clinical Corynebacteria to Macrolide, Lincosamide and Streptogramin B

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Submitted 16 October 2017, revised 7 February 2018, accepted 20 February 2018

Abstract

The subject of the study was phenotypic marking of the antibiotic susceptibility and MLSB resistance mechanism in Corynebacterium spp. isolated from human skin (18 isolates) and from clinical materials (19 isolates). The strains were tested for the presence of the erm(A), erm(B), erm(C), erm(X), lnu(A), msr(A), msr(B) and mph(C) genes. Clinical isolates showed wide resistance to antibiotics. In 89% clinical isolates and 72% skin microbiota a constitutive type of MLSB resistance was found. In 12 clinical isolates the erm(C) gene was detected-eight of which had erm(X) as well as erm(C), two harboured erm(X), erm(C) and erm(A) and two demonstrated only erm(C).

Key words: Corynebacterium spp., erm(C), MLSB resistance genes

Escalating resistance of bacteria to antibiotics, including macrolides, leads to progressive difficulties in treatment of bacterial infections. The problems also concern infections caused by opportunistic corynebacteria (Ortiz-Perez et al., 2010; Olinger, 2013; Alibi et al., 2007). Resistance to macrolides often results from the enzymatic methylation of adenosine of the 23S rRNA ribosomal subunit and is encoded by the genes from the erm family. The above-described mechanism affects resistance to lincosamides and streptogramin B and so that it is referred to as the MLSB (Maravic, 2004). Phenotypic expression of MLSB resistance can be constitutive or inducible. Strains with constitutive MLSB (cMLSBB) resistance are considered resistant to all macrolides, lincosamides and streptogramin B, whereas strains with inducible MLSB (iMLSBB) present resistance to 14- and 15-member macrolides but appear susceptible to 16-member macrolides, lincosamides and type B streptogramins (Weisblum, 1995). Detection of any of these mechanisms eliminates such groups of antibiotics from the therapy. Additionally, resistance to macrolides, lincosamides and streptogramin B may also be caused by other mechanisms such as efflux pumps or enzymatic inactivation of antibiotics encoded by other groups of genes (Roberts et al., 1999; Roberts, 2008).

Resistance to macrolides, lincosamides, and streptogramin B in Corynebacterium spp. is noticeable especially in clinical multidrug-resistant isolates (Otsuka et al., 2006; Ortiz-Perez et al., 2010). Nevertheless, in a manuscript published in Microbial Drug Resistance in 2014 (Szemraj et al., 2014), we have shown that this resistance may also occur in the skin microbiota e.g. C. tuberculostearicum or C. jeikeium. Our study covered 99 strains and confirmed earlier reports that the gene responsible for this mechanism in corynebacteria is erm(X). During that study we did not find other genes whereas other researchers demonstrated existence of the erm(B), mef or msr(A) genes in these bacteria (Luna et al., 1999; Ojo et al., 2006; Ortiz-Perez et al., 2010).

In the following years we pursued the studies on new groups of strains. The aim of the research was phenotypic marking of the antibiotic susceptibility and detecting of MLSB resistance mechanism in Corynebacterium spp. For the investigation we used 18 strains from skin of healthy young men that did not have long-term contact with the healthcare services (natural microbiota) and 19 strains from clinical materials (mainly from blood, urine and wounds).

The strains were identified by the MALDI-TOF/VITEK MS mass spectrometry system (bioMerieux), performed in the Synevo laboratory in Łódź, and by the Coryne API (bioMerieux) phenotypic method. Among the clinical isolates and skin microbiota tested there were lipophilic as well as non-lipophilic species. Sensitivity to penicillin, tetracycline, erythromycin, clindamycin, gentamicin, ciprofloxacin, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethoprim/sulfamethoxazole, trimethyl...
tigecycline and linezolid was being marked by the disc diffusion method according to the EUCAST guidelines. S. pneumoniae ATCC 49619 was used as a control strain. In the breakpoints absence, CLSI guidelines for Corynebacterium spp. were used. When it was not possible to apply such criteria, EUCAST guidelines were administered as for Staphylococcus spp. Both the inducible and constitutive resistance mechanisms were phenotypically determined in compliance with the EUCAST guidelines for Staphylococcus species by the application of S. aureus ATCC 29213 as the control strain. DNA was isolated with the usage of Genomic Mini (A&A Biotechnology) commercial set in line with the manufacturer’s protocol. PCR was performed by the application of the primers (Genomed, Sequencing Laboratory, Poland) specific for the erm(A), erm(B), erm(C) (Sutcliffe et al., 1996), erm(X) (Rosato et al., 2001), mph(C) (Perreten et al., 2005), msr(A) (Ojo et al., 2006), msr(B) and lnu(A) (Lina et al., 1999) genes. DNA amplification was carried out in the thermal cycler (Biometra). PCR were initialized by a denaturation step (5 min at 94°C), followed by 30 cycles of amplification (denaturation 30 sec at 94°C, annealing 30 sec at 46–56°C, DNA chain extension 1 min at 72°C), and a final extension step (10 min at 72°C). Reaction products were recognized by electrophoresis (70V by 1 h) in 1% (w/v) agarose gels with Midori Green DNA Stain (NIPPON Genetics EUROPE). For the readout of the outcomes the CCD camera (Syngen) was used. Due to a commercial molecular size marker the amplification products sizes were determined using DraMix (A&A BIOTECHNOLOGY). C. jeikeium K411 (CCUG 27385) harbouring erm(X) and S. epidermidis strains: 3718INL harbouring erm(B) and mph(C), 1486FG with erm(A), erm(C) and mph(C) and 1923KIINL with the msr(A) and lnu(A) genes were used as positive controls (Juda, 2010).

Similarly to the former work, the identification of strains occurred to be tough. However, by the usage of MALDI-TOF mass spectrometry (that was based on the analysis of the protein profiles of the tested strains) and phenotypic methods we managed to identify most of the tested strains i.e. 17 clinical isolates and 13 isolates of skin microbiota. None of genetic methods was applied. For the Corynebacterium spp., the sequencing of the 16S rRNA subunit gene limits the low polymorphism of such sequence between species. Thus, sequencing the rpoB gene is the only possibility (Khamis et al., 2004). Among clinical isolates C. striatum and C. jeikeium prevailed, while among the skin microbiota C. tubercidostearicum and C. jeikeium predominated. C. jeikeium, similarly to other corynebacteria, is often described as the cause of infection and less often as a sample contamination (Wang et al., 2001; Akan et al., 2002; Mookadam et al., 2006). C. jeikeium and C. striatum were isolated from endocarditis, bones, joints, lungs, cerebrospinal and bacteremia (Lee et al., 2005; Tleyjeh et al., 2005; Belmares et al., 2007; Chen et al., 2012; Daisuke et al., 2017).

The clinical isolates (especially C. striatum, C. amycolatum and C. jeikeium) showed a wide resistance to antibiotics. All strains from this group were resistant to at least three antibiotics (Fig. 1), nine strains to six antibiotics and five to seven antibiotics (three strains of C. striatum, one of C. amycolatum/xerosis and one of C. glucuronolyticum).

Soriano et al. (1995), Reddy et al. (2012) and Hahn et al. (2016) also pointed out the growing resistance of corynebacteria isolated from clinical materials. Among the skin microbiota five strains were sensitive to all tested antibiotics whereas the others were resistant to at least two up to six antibiotics. All strains from both groups were sensitive to linezolid and tigecycline. Similar Corynebacterium spp. sensitivity to these antibiotics was also presented by Morata et al. (2014), Tang et al. (2015) and Alibi et al. (2007). The constitutive type of MLS$_{B}$ resistance was demonstrated in 17 strains tested from clinical specimens (89%) while the other two iso-

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**Fig. 1.** Percentage of strains resistant to two or more antibiotics among skin microbiota and clinical isolates.
lates were resistant to one from two antibiotics. This resistance was accompanied by the \textit{erm(A), erm(C)} and \textit{erm(X)} genes – individually or in groups. The \textit{erm(B), msr(A), msr(B), mph(C)} and \textit{lau(A)} genes were not found in the genomes of any of the strains tested. The constitutive MLS\textsubscript{S} resistance mechanism was also demonstrated in 13 resistant strains isolated from healthy human skin (72%). These strains were harbouring only the \textit{erm(X)} gene what has confirmed our previous observations (Szemraj et al., 2014).

It draws the attention that we detected for the first time a large number of clinical isolates of \textit{Corynebacterium} spp. which alongside with the commonly described genes such as \textit{erm(X)} (Olender and Niemcewicz, 2010; Olender, 2013) were harbouring also \textit{erm(C)}. In two isolates, three genes: \textit{erm(X)}, \textit{erm(C)} and \textit{erm(A)} simultaneously existed. In other two isolates (the first resistant to macrolide and lincosamides and the second only to macrolide), solely \textit{erm(C)} was detected (Table I).

These results indicate the importance of this gene in the spreading of MLS\textsubscript{S} resistance mechanism in \textit{Corynebacterium} spp. which until now has not been frequently sought in corynebacteria in spite of the fact that it is often found in staphylococci (Chaieb et al., 2007; Gatermann et al., 2010). Our finding of the \textit{erm(C)} gene among \textit{Corynebacterium} spp. that is responsible for the MLS\textsubscript{S} mechanism mainly in coagulase-negative staphylococci, indicates the potential for gene acquisition by clinical strains from these bacteria (Chaieb et al., 2007; Gatermann et al., 2007; Cetin et al., 2010).

| Resistance phenotype | Resistance genes (number of isolates and species) | Clinical isolates | Skin microbiota |
|----------------------|--------------------------------------------------|------------------|-----------------|
|                      | \textit{erm(X)}, \textit{erm(C)}, \textit{erm(X)+erm(C)}, \textit{erm(X)+erm(C)+erm(A)} | C. striatum | C. jeikeium |
|                      | \textit{erm(X)}, \textit{erm(C)}, \textit{erm(X)+erm(C)}, \textit{erm(X)+erm(C)+erm(A)} | C. glaucorosyniticum | C. jeikeium |
|                      | \textit{erm(X)}, \textit{erm(C)}, \textit{erm(X)+erm(C)}, \textit{erm(X)+erm(C)+erm(A)} | C. amycolatum/xerosis | C. tuberculostearicum |
| Clinical isolates    |                                                  | C. aurimucosum | Corynebacterium sp. |
| E\textsuperscript{C}\textsuperscript{S} | 6 1 8 2 | 13 0 0 0 | 0 0 |
| E\textsuperscript{C}\textsuperscript{C} | 0 1 0 0 | 0 0 1 0 | 0 0 |
| Skin microbiota      |                                                  | 0 0 1 0 0 0 0 0 0 0 |

E\textsuperscript{C}\textsuperscript{S} – resistant both to erythromycin and clindamycin, E\textsuperscript{C}\textsuperscript{C} – resistant to erythromycin and susceptible to clindamycin, E\textsuperscript{C}\textsuperscript{C} – susceptible to erythromycin and resistant to clindamycin.

Table I

**Distribution of resistance genes among clinical isolates and skin microbiota.**

Acknowledgments

This study was supported by grants 502-03/3-012-03/502-34-098 from the Medical University of Łódź.

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