

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

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Abstract

The subject of the study was phenotypic marking of the antibiotic susceptibility and MLS_B 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 MLS_B 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), MLS_B, 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; Olender, 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 MLS_B (Maravic, 2004). Phenotypic expression of MLS_B resistance can be constitutive or inducible. Strains with constitutive MLS_B (cMLS_B) resistance are considered resistant to all macrolides, lincosamides and streptogramin B, whereas strains with inducible MLS_B (iMLS_B) 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 MLS_B 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 (bioMérieux), performed in the Synevo laboratory in Łódź, and by the Coryne API (bioMérieux) 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,

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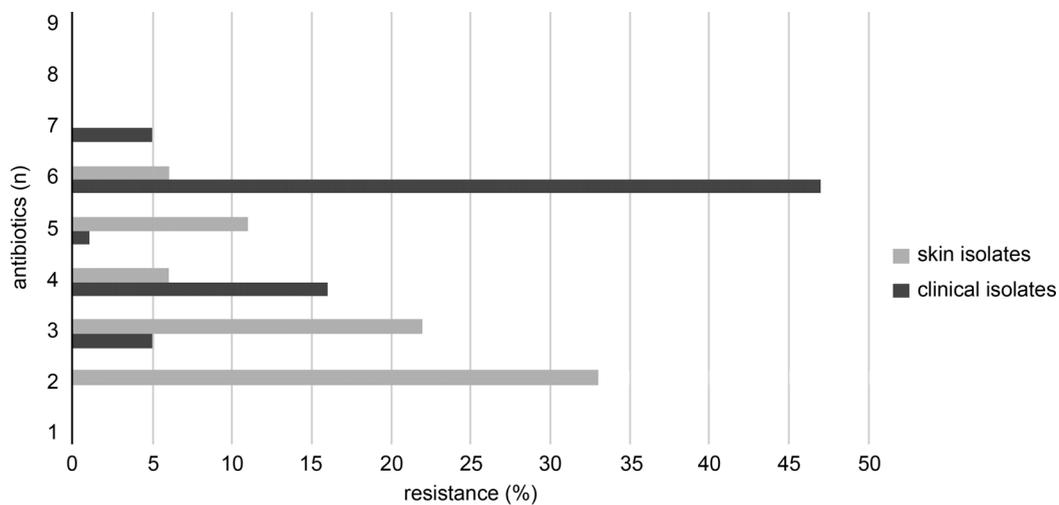


Fig. 1. Percentage of strains resistant to two or more antibiotics among skin microbiota and clinical isolates.

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 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), 1486IG 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. tuberculo-stearicum* 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-

Table I
Distribution of resistance genes among clinical isolates and skin microbiota.

Resistance phenotype	Resistance genes (number of isolates and species)			
	<i>erm(X)</i>	<i>erm(C)</i>	<i>erm(X)+erm(C)</i>	<i>erm(X)+erm(C)+erm(A)</i>
Clinical isolates	<i>C. striatum</i> <i>C. glucuronolyticum</i> <i>C. amycolatum/xerosis</i>	<i>C. aurimucosum</i>	<i>C. jeikeium</i> <i>C. striatum</i> <i>C. aurimucosum</i>	<i>C. pseudituberculosis</i> <i>Corynebacterium</i> sp.
E ^R C ^R	6	1	8	2
E ^R C ^S	0	1	0	0
E ^S C ^R	0	0	1	0
Skin microbiota	<i>C. jeikeium</i> <i>C. tuberculostearicum</i> <i>Corynebacterium</i> sp.			
E ^R C ^R	13	0	0	0

E^RC^R – resistant both to erythromycin and clindamycin, E^RC^S – resistant to erythromycin and susceptible to clindamycin, E^SC^R –susceptible to erythromycin and resistant to clindamycin

lates were resistant to one from two antibiotics. This resistance was accompanied by the *erm(A)*, *erm(C)* and *erm(X)* genes – individually or in groups. The *erm(B)*, *msr(A)*, *msr(B)*, *mph(C)* and *lnu(A)* genes were not found in the genomes of any of the strains tested. The constitutive MLS_B resistance mechanism was also demonstrated in 13 resistant strains isolated from healthy human skin (72%). These strains were harboring only the *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 *Corynebacterium* spp. which alongside with the commonly described genes such as *erm(X)* (Olender and Niemcewicz, 2010; Olender, 2013) were harbouring also *erm(C)*. In two isolates, three genes: *erm(X)*, *erm(C)* and *erm(A)* simultaneously existed. In other two isolates (the first resistant to macrolide and lincosamides and the second only to macrolide), solely *erm(C)* was detected (Table I).

These results indicate the importance of this gene in the spreading of MLS_B resistance mechanism in *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; Cetin *et al.*, 2010).

The results obtained show that clinical strains derived from the strains accounting for natural microbiota easily acquire new antibiotic resistance genes. The fact mentioned above was proven by the resistance profile of the strains tested including genes that provide resistance to MLS_B antibiotics. There is a possibility of horizontal gene transfer in these bacteria what can be suggested by the different location of the *erm(X)* gene in the *Corynebacterium* spp. In *C. diptheriae* it is a part of 14.5 kbp the plasmid pNG2, in *C. xerosis* this gene is a part of the Tn5432 transposon located on the 50 kbp Ptp10 plasmid, whereas in *C. jeikeium* and *C. striatum* it is often located on the Tn5432 transposon inside the

chromosome (Roberts *et al.*, 1992; Tauch *et al.*, 1995, 2003, 2005; Rosato *et al.*, 2001). Tn5432 transposon harboring *erm(X)* is also found in other *Propionibacterium* spp. (Ross *et al.*, 2002; El-Mahdy *et al.*, 2010). Our finding of the *erm(C)* gene among *Corynebacterium* spp. that is responsible for the MLS_B 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).

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Literature

- Akan A., J. Bengisun, A. Bati, M. Arat, O. Ilhan and H. Akan. 2002. A case of catheter related septicemia caused by *Corynebacterium jeikeium*. *Turk. J. Haematol.* 19: 47–50.
- Alibi S., A. Ferjani, J. Boukadida, M.E. Cano, M. Fernández-Martínez, L. Martínez-Belmares J., S. Detterline, J.B. Pak and J.P. Parada. 2007. *Corynebacterium* endocarditis species – specific risk factors and outcomes. *BMC Infect. Dis.* 7: 4.
- Cetin E.S., H. Gunes, S. Kaya, B.C. Aridogan and M. Demirci. 2010. Distribution of genes encoding resistance to macrolides, lincosamides and streptogramins among clinical staphylococcal isolates in a Turkish University Hospital. *J. Microbiol. Immunol. Infect.* 43: 524–529.
- Chaieb K., T. Zmantar, O. Chehab, O. Bouchami, A.B. Hasen and K. Mahdouani. 2007. Antibiotic resistance genes detected by multiplex PCR assays in *Staphylococcus epidermidis* strains isolated from dialysis fluid and needles in a dialysis service. *Jpn. J. Infect. Dis.* 60: 183–187.
- Chen F.L., P.R. Hsueh, S.O. Teng, T.Y. Ou, W.S. Lee 2012. *Corynebacterium striatum* bacteremia associated with central venous catheter infection. *J. Microbiol. Immunol. Infect.* 45: 255–258.
- CLSI, Clinical and Laboratory Standards Institute. 2015. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline – 3rd ed. M45, Wayne, PA, USA.

- Daisuke U., T. Oishi, K. Yamane and K. Terada. 2017. *Corynebacterium striatum* bacteremia associated with a catheter-related blood stream infection. 2017. *Case Rep. Infect. Dis.* 2017: 2682149.
- El-Mahdy T.S., S. Abdalla, R. El-Domany, M.S. Mohamed, J.I. Ross and A.M. Snelling. 2010. Detection of a new *erm(X)*-mediated antibiotic resistance in Egyptian cutaneous propionibacteria. *Anaerobe* 16: 376–379.
- EUCAST, European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, valid from 2017.03.13.
- Gatermann S.G., T. Koschinski and S. Friedrich. 2007. Distribution and expression of macrolide resistance genes in coagulase-negative staphylococci. *Clin. Microbiol. Infect.* 13: 777–781.
- Hahn W. O., J. B. Werth, S. M. Butler-Wu and R. M. Rakita. 2016. Multidrug-resistant *Corynebacterium striatum* associated with increased use of parenteral antimicrobial drugs. *Emerg. Infect. Dis.* 22: 1908–1914.
- Juda M. 2010. PhD. thesis, Phenotypic and genetic analysis of coagulase-negative staphylococci resistance to macrolides, lincosamides and streptogramins (in Polish). Department of Pharmaceutical Microbiology with Laboratory for Microbiological Diagnostics, Medical University of Lublin. Lublin. Poland.
- Khamis A., D. Raoult and B. La Scola. 2004. *rpoB* gene sequencing for identification of *Corynebacterium* species. *J. Clin. Microbiol.* 42: 3925–3931.
- Lee P.P., D.A. Ferguson and F.A. Sarubbi. 2005. *Corynebacterium striatum*: an underappreciated community and nosocomial pathogen. *J. Infect.* 50: 338–343.
- Lina G., A. Quaglia, M. Reverdy, R. Leclercq, F. Vandenesch, and J. Etienne. 1999. Distribution of genes encoding resistance to macrolides, lincosamides and streptogramins among staphylococci. *Antimicrob. Agents Chemother.* 43: 1062–1066.
- Luna V.A., P. Coates, A. Eady, J.H. Cove., T.T.H. Nguyen and M.C. Roberts. 1999. A variety of Gram-positive bacteria carry mobile *mef* genes. *J. Antimicrob. Chemother.* 44: 19–25.
- Maravic G. 2004. Macrolide resistance based on the Erm-mediated rRNA methylation. *Curr. Drug Targets Infect. Disord.* 4: 193–202.
- Mookadam F., M. Cikes, L.M. Baddour, I.M. Tleyjeh and M. Mookadam. 2006. *Corynebacterium jeikeium* endocarditis: a systematic overview spanning four decades. *Eur. J. Clin. Microbiol. Infect. Dis.* 25: 349–353.
- Morata L., E. Tornero, J.C. Martínez-Pastor, S. García-Ramiro, J. Mensa and A. Soriano. 2014. Clinical experience with linezolid for the treatment of orthopaedic implant infections. *J. Antimicrob. Chemother.* 69: 47–52.
- Ojo K.K., M.J. Striplin, C.C. Ulep, N.S. Close, J. Zittle, H. Luis, J. Leitao, M.C. Roberts and M. Bernardo. 2006. Staphylococcus efflux *msr(A)* gene characterized in *Streptococcus*, *Enterococcus*, *Corynebacterium*, and *Pseudomonas* isolates. *Antimicrob. Agents Chemother.* 50: 1089–1091.
- Olender A. 2013. Antibiotic resistance and detection of the most common mechanism of resistance (MLSB) of opportunistic *Corynebacterium*. *Chemotherapy* 59: 294–306.
- Olender A. and M. Niemcewicz. 2010. Macrolide, lincosamide, and streptogramin B-constitutive-type resistance in *Corynebacterium pseudodiphtheriticum* isolated from upper respiratory tract specimens. *Microb. Drug Resist.* 16: 119–122.
- Ortiz-Perez A., N.Z. Martin-de-Hijas, J. Esteban, M.I. Fernandez-Natal, J.I. Garcia-Cia and R. Fernandez-Roblas. 2010. High frequency of macrolide resistance mechanisms in clinical isolates of *Corynebacterium* species. *Microb. Drug Resist.* 16: 273–277.
- Otsuka Y., K. Ohkusu, Y. Kawamura, S. Baba, T. Azeki and S. Kimura. 2006. Emergence of multidrug-resistant *Corynebacterium striatum* as a nosocomial pathogen in long-term hospitalized patients with underlying diseases. *Diagn. Microbiol. Infect. Dis.* 54: 109–114.
- Perreten V., L. Vorlet-Fawer, P. Slickers, R. Ehrlich, P. Kuhnert, and J. Frey. 2005. Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. *J. Clin. Microbiol.* 43: 2291–2302.
- Reddy B.S., A. Chaudhury, U. Kalawat, R. Jayaprada, G.S.K. Reddy and B.V. Ramana. 2012. Isolation, speciation and antibiogram of clinically relevant non-diphtherial *Corynebacteria* (Diphtheroids). *Indian J. Med. Microbiol.* 30: 52–57.
- Roberts M.C. 2008. Update on macrolide-lincosamide streptogramin, ketolide, and oxazolidinone resistance genes. *FEMS Microbiol. Lett.* 282: 147–159.
- Roberts M.C., R.B. Leonard, A. Briselden, F.D. Schoenkecht and M.B. Coyle. 1992. Characterization of antibiotic-resistant *Corynebacterium striatum* strains. *J. Antimicrob. Chemother.* 30: 463–474.
- Roberts M.C., J. Sutcliffe, P. Courvalin, L.B. Jensen, J. Rood, and H. Seppala. 1999. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob. Agents Chemother.* 43: 2823–2830.
- Rosato A.E., B.S. Lee and K.A. Nash. 2001. Inducible macrolide resistance in *Corynebacterium jeikeium*. *Antimicrob. Agents Chemother.* 45: 1982–1989.
- Ross J.I., A.A. Eady, E. Carnegle and J.H. Cove. 2002. Detection of transposon Tn5432-mediated macrolide-lincosamide-streptogramin B (MLSB) resistance in cutaneous propionibacteria from six European cities. *J. Antimicrob. Chemother.* 49: 165–168.
- Soriano F., J. Zapardiel and E. Nieto. 1995. Antimicrobial susceptibilities of *Corynebacterium* species and other non-spore-forming gram-positive bacilli to 18 antimicrobial agents. *Antimicrob. Agents Chemother.* 39: 208–214.
- Sutcliffe J., T. Grebe, A. Tait-Kamradt, and L. Wondrack. 1996. Detection of erythromycin-resistant determinants by PCR. *Antimicrob. Agents Chemother.* 40: 2562–2566.
- Szemraj M., A. Kwaszewska, R. Pawlak and E.M. Szweczyk. 2014. Macrolide, lincosamide, and streptogramin B resistance in lipophilic corynebacteria inhabiting healthy human skin. *Microb. Drug Resist.* 20: 404–409.
- Tang S., L. Yao, X. Hao, X. Zhang, G. Liu, X. Liu, M. Wu, L. Zen, H. Sun, Y. Liu and others. 2015. Efficacy, safety and tolerability of linezolid for the treatment of XDR-TB: a study in China. *Eur. Respir. J.* 45: 161–170.
- Tauch A., N. Bischoff, I. Brune and J. Kalinowski. 2003. Insights into the genetic organization of the *Corynebacterium diphtheriae* erythromycin resistance plasmid pNG2 deduced from its complete nucleotide sequence. *Plasmid.* 49: 63–74.
- Tauch A., O. Kaiser, T. Hain, A. Goesmann, B. Weisshaar, A. Albersmeier, T. Bekel, N. Bischoff, I. Brune, T. Chakraborty and others. 2005. Complete genome sequence and analysis of the multidrug-resistant nosocomial pathogen *Corynebacterium jeikeium* K411, a lipid-requiring bacterium of the human skin flora. *J. Bacteriol.* 187: 4671–4682.
- Tauch A., F. Kassing, J. Kalinowski and A. Pühler. 1995. The *Corynebacterium xerosis* composite transposon Tn5432 consists of two identical insertion sequences, designated IS1249, flanking the erythromycin resistance gene *ermCX*. *Plasmid* 34: 119–131.
- Tleyjeh I.M., M.O. Qutub, M. Bakleh, M.R. Sohail and A. Virk. 2005. *Corynebacterium jeikeium* prosthetic joint infection: case report and literature review. *Scand. J. Infect. Dis.* 37: 151–153.
- Wang C., D. Mattson and A. Wald. 2001. *Corynebacterium jeikeium* bacteremia in bone marrow transplant patients with Hickman catheters. *Bone Marrow Transplant* 27: 445–449.
- Weisblum B. 1995. Erythromycin resistance by ribosome modification. *Antimicrob. Agents Chemother.* 39: 577–585.



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