

Flow-FISH as an Estimator of Erythromycin Resistance in Enterococcal Isolates from Rtx Patients

Tomasz Jarzembowski¹, Agnieszka Daca²

¹Department of Microbiology, Medical University of Gdańsk Poland

²Department of Pathology and Experimental Rheumatology, Medical University of Gdańsk Poland

*Corresponding Author: Tomasz Jarzembowski, Department of Microbiology, Medical University of Gdańsk Poland

ABSTRACT

Macrolide-lincosamide-streptogramin (MLS) antibiotics are still an alternative therapy for the insidious enterococcal infection. However, resistance to macrolides increase and therefore an investigation into the erythromycin-resistance has become imperative. The most common mechanism of resistance is the target modification to the binding site of macrolides, lincosamides, and streptogramin B antibiotics mediated by Erm – rRNA methylase alters a site in 23S rRNA. In current study authors evaluate the detection of *ermB* gene at a single cell level by Flow-FISH technique. Thirty six enterococcal strains were isolated from urine of RTx patients hospitalized at Medical University of Gdansk.

The MIC value for all isolates varied from 0,25 to above 256 µg/ml. 22 out of 36 strains were found to be resistant to erythromycin. Coincidence of phenotypic and genotypic results confirm reasonability of FLOW-FISH attitude: the difference between resistant and susceptible strains was significant at $p = 0,037$.

Keywords: macrolide resistance, enterococci, FLOW-FISH, RTx

INTRODUCTION

Over the last few years, enterococci became an important etiological agent of nosocomial infections. The acquisition of resistance gene to different antibiotics makes infections with these microorganisms difficult to treat. Macrolide-lincosamide-streptogramin (MLS) antibiotics are still an alternative therapy for the insidious enterococcal infection (1). Erythromycin, a macrolides antibiotic, is effective against a variety of gram positive bacteria and commonly used. However, resistance to macrolides increase and therefore an investigation into the erythromycin-resistance has become imperative (2).

So far three different mechanisms have been described. The most common is target modification to the binding site of macrolides, lincosamides, and streptogramin B antibiotics mediated by Erm- rRNA methylase alters a site in 23S rRNA. The second mechanism involves enzymes (EreA and EreB) that hydrolyze the lactone ring of the macrolides. Additionally, the phosphotransferases inactivate macrolides by introducing a phosphate on the 29-hydroxyl group of the amino sugar in rods of the family Enterobacteriaceae and gram positive cocci *Staphylococcus aureus*. The presence of multicomponent macrolides efflux pumps in

staphylococci (*msrA*, *msrB*) as well as an efflux system in streptococci (*mefA*, *mefE*) has also been documented (3).

The bacterial antibiotic resistance may be successfully determined by flow cytometry (4). This is a technique that permits rapid analysis of individual cells and allows quantitative evaluation of the distribution of some properties in a population. Thus detection of hetero resistance of the strains. In our previous study, fluorescent antibiotics were successfully used for determination of bacterial resistance. Here we aimed to evaluate Flow-FISH technique to screen the prevalence of common erythromycin resistance gene *ermB* in enterococcal isolates.

MATERIAL AND METHODS

Thirty six enterococcal strains were isolated from urine of RTx patients hospitalized at Medical University of Gdansk. The isolates were identified to species level by strep ID test (BioMerieux) and classified as different strains of *Enterococcus faecalis* by biochemical and resistance profiles. Phenotypic resistance to erythromycin was determined by disc-diffusion method and by E-test method. To evaluate gene expression using the Flow-FISH method, hybridization procedure described by Waar et al. (4) was adopted and modified (5). Briefly, cell

membranes were permeabilized by incubation for 30min at 37°C in permeabilisation buffer (Tris-EDTA) containing 1mg/ml lysozyme (DNA Gdansk, Poland). Then, the cells were suspended in 1mL of 0.9%NaCl and sonicated for 2min on ice. The sequence of the probe to detect ermB gene:

TTGCTCTTGCACACTCAAGTCTCGATTCA was designed by the commercial software (primer express, Applied Biosystems, USA), synthesized and labeled with the Cy3 commercially by Genomed Company, Poland. The probe was hybridized at Tm temperature (45°C) for 60 min. To ensure permeabilisation of the cells, propidium iodide (PI, 1 µg/mL) staining of DNA was performed. Particles without PI fluorescence (FL3) were excluded from further investigation. Fluorescence of particles was determined using FACScan flow cytometer (Becton-Dickinson, Franklin Lakes, NJ, USA). The mean probe fluorescence was normalized by total DNA fluorescence. Results were tested by analysis of variance (ANOVA) by Stat Soft software, Poland (Statistica 12).

RESULTS AND DISCUSSION

Enterococci are among the most common pathogen of urinary tract infection (UTI). They may also be a leading pathogen of UTI after kidney transplantation (7). The immunosuppression in such patients makes resistance of enterococci to antibiotic especially important. 22 out of 36 studied strains were found to be resistant to erythromycin in disk diffusion determination. The MIC value for all isolates varied from 0,25 to above 256 µg/ml. Such results could be expected as similar results were reported for other European countries (2). The median fluorescence of the probe was 6,16 for resistant strains (1,21- 64,1) and 2,83 for the phenotypically susceptible. The difference between resistant and

susceptible strains was significant at p = 0,037. Coincidence of phenotypic and genotypic results confirm reasonability of our method (Figure 1). On the other hand the high diversity of probe signal and MIC value strongly suggest the existence of other than ermB mediated mechanisms of resistance in case of five studied strains (Figure 2). The ermB gene has previously been demonstrated to be involved in macrolides resistance in different gram-positive bacteria, such as *Enterococcus* (2). What is more important, the results of other authors indicate that *erm* genes are present only in highly macrolides-resistant strains of *Enterococcus* (1). Also all of our data indicate that the *ermB* gene is most frequently found among the highly resistant Enterococcus isolates, thus, its acquisition could have a predominant role in the development of high-level erythromycin resistance in Enterococcus spp. (1). It is also essential to consider the phenomenon of erythromycin heteroresistance of the strains described recently (6). Such mechanism could be responsible for the differences of probe binding (Figure 2). Unfortunately at this stage there is no evidence available and the further studies are required.

The last but not least aspect that should be considered is the effect of immunotherapy on expression of resistance genes in enterococci. Such phenomenon has been already described in relation to PBP expression (5) and the influence of calcineurin inhibitors on PBP expression has been proved. No doubt, the choice of immunosuppressant may have serious influence not only at risk of infection but also on prevalence of antibiotic resistance. However, the analysis of influence on immunosuppressant used in RTx patients on erythromycin resistance gene expression was beyond the scope of the current study.

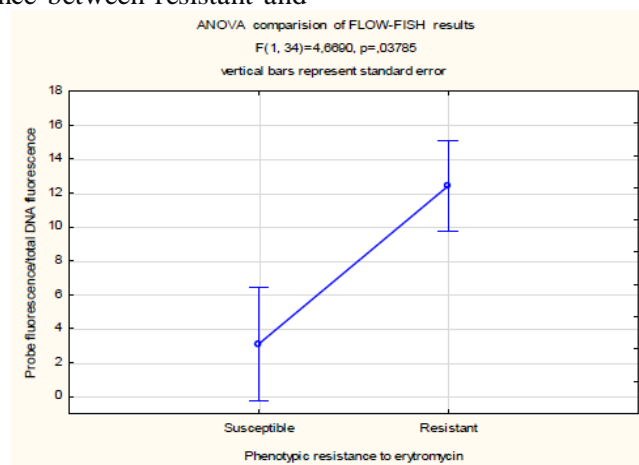


Figure1. Value of estimators for the phenotypically resistant and susceptible strains

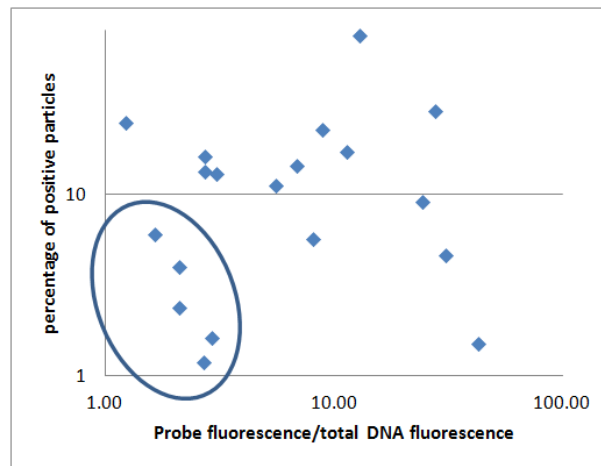


Figure 2. Distribution of probe signal for erythromycin resistant strains

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