

Is the mazEF toxin-antitoxin system responsible for vancomycin resistance in clinical isolates of *Enterococcus faecalis*?

Ist das mazEF Toxin-Antitoxin-System verantwortlich für die Vancomycinresistenz klinischer Isolate von *Enterococcus faecalis*?

Abstract

The current study was conducted to investigate the relationship between vancomycin-resistant *Enterococcus faecalis* (VRE) and the presence of *mazEF* toxin-antitoxin (TA) system, which may be useful as target for novel antimicrobial therapy concepts. The susceptibility of *E. faecalis* was determined by MIC, and the presence of the *mazEF* TA system was evaluated by PCR.

Among 200 *E. faecalis* isolates 39.5% showed resistance to vancomycin (VRE), while 60.5% were susceptible strains (VSE). The *mazEF* TA system was positive in all VRE isolates (100%), but less prevalent (38/121, 31.4%) among the 121 VSE strains.

In conclusion, our study demonstrated a positive relationship between the presence of vancomycin resistance and *mazEF* TA system. This observation may introduce therapeutic options against a novel antimicrobial target in enterococci.

Keywords: Enterococcus, *mazEF* TA system, vancomycin, antimicrobial target

Zusammenfassung

In der vorliegenden Studie sollte der Zusammenhang zwischen der Vancomycinresistenz bei *Enterococcus faecalis* (VRE) und dem Vorkommen des *mazEF* Toxin-Antitoxin(TA)-Systems untersucht werden, um Hinweise für eine neue antimikrobielle Targettherapie zu erhalten. Die Empfindlichkeit der Enterokokken wurde mittels MIC, das Vorkommen des *mazEF* TA-Systems mittels PCR bestimmt.

Unter 200 Enterokokkenisolaten waren 39,5% resistent gegen Vancomycin (VRE), während 60,5% sensibel waren. Das *mazEF* TA-System war bei allen VRE-Isolaten positiv, aber weniger prävalent bei den VSE-Isolaten (38/121, 31,4%).

Die Analyse ergab einen Zusammenhang zwischen dem Vorkommen des VRE-Resistenzgens und dem *mazEF* TA-System, was für weitere Untersuchungen in Hinblick auf ein neues antimikrobielles Ziel gegenüber Enterokokken hilfreich ist.

Schlüsselwörter: Enterococcus, *mazEF* AT-System, Vancomycin, antimikrobielles Target

Introduction

Enterococcus is one of the important organisms which is responsible for clinical infections such as urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis [1]. *Enterococcus* antibiotic sensitive strains can be treated with β -lactam antibiotics such as

ampicillin and glycopeptides such as vancomycin [2]. Regretfully, resistance against antibiotics is increasing globally [3]. In the last two decades, vancomycin resistance of enterococci has emerged increasingly in nosocomial infections of hospitalized patients [1]. Mobile genetic elements are responsible for the vancomycin resistance in enterococci [4]. Despite the frequency

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of plasmid resistance among vancomycin-resistant enterococci (VRE) isolates is not obvious, there are multiple reports of plasmids with the various vancomycin resistant genes clusters. Some plasmids harbor loci encoded post-segregation killing systems [5], which encode a toxin and its corresponding antitoxin. The analysis showed that this system could be vertically transferred. The antitoxin is unstable; however, the toxin is stable, which adds to post-segregation killing systems [6]. Interestingly, the stable toxin may trigger bacteriostasis among VRE strains. The current study was conducted to explain the relationship between vancomycin resistance of *Enterococcus faecalis* and the presence of *mazEF* TA system, which may be useful as target for novel antimicrobial therapy concepts.

Methods

Bacterial isolates and identification

Two hundred isolates of *Enterococcus faecalis* were collected during September 2011 and April 2012 in Ilam Hospital in the West of Iran and Milad Hospital in Tehran, the capital of Iran. The isolates were obtained from patients with urinary tract infection. The *Enterococcus faecalis* identification was performed by Gram staining, motility assessment, catalase production, growth in 6.5% NaCl, xylose, mannitol, arabinose, sorbitol use, bile, and esculin growth. Strains were additionally tested for hydrolysis, pigment production, leucine aminopeptidase activity, and acidification of methyl-a-D-glucopyranoside [7].

Determination of vancomycin-resistant *Enterococcus* (VRE)

Minimum inhibitory concentrations (MICs) were assessed by microdilution in Mueller-Hinton broth. MICs of vancomycin were defined as being resistant to vancomycin with an MIC ≥ 8 $\mu\text{g}/\text{mL}$ [8]. *E. faecalis* ATCC 51299 was used as positive control [8].

Determination of the MazEF TA system

The specific primers were designed for *mazEF* TA loci to amplify 505 bp oligonucleotide. The primer sequences of PCR primers were as follows: Forward: 5-ATGATCCACAGTAGCGTAAAGCGT-3; Reverse: 5-TTACCAGACTTCCTTATCTTTCCGG-3.

The PCR amplification was carried out in a final volume of 25 μl with 3 μl of DNA as a template, 2.5 μl PCR buffer (20 mM Tris-HCl/50 mM KCl, pH 8.4), 1.5 mM MgCl₂, 1 mM each deoxynucleoside triphosphate, 1 μM each primer, and 2 units of *Taq* polymerase. The PCR was performed with an initial denaturation in 95 °C for 2 minutes and 35 cycles of denaturation in 94 °C for 1 minute, annealing in 58 °C for 45 seconds, and extension in 72 °C for 30 seconds, following in a final extension

step in 72 °C for 10 minutes. Then, the PCR products were analyzed by 1% agarose gel electrophoresis.

Results

Prevalence of VRE

Among the 200 *E. faecalis* isolates (Table 1), 39.5% of the isolates showed resistance to vancomycin (VRE), while 60.5% were susceptible to vancomycin (VSE) (Figure 1).

Table 1: Prevalence of VRE in Milad and Ilam Hospitals

| | <i>Enterococcus</i> | VSE | VRE |
|-----------------------|---------------------|-------------|------------|
| Milad Hospital | 110 (55%) | 48 (43.7%) | 62 (56.3%) |
| Ilam Hospital | 90 (45%) | 73 (81.1%) | 17 (18.9%) |
| Total | 200 (100%) | 121 (60.5%) | 79 (39.5%) |

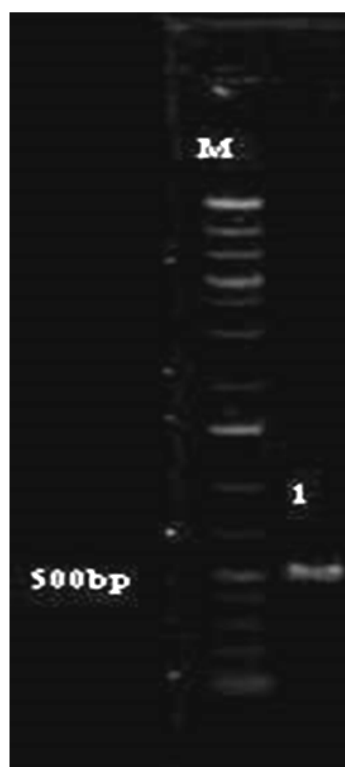


Figure 1: PCR analysis of VRE clinical isolates; M (Marker 1 kb plus, Thermo Science), 1 = *mazEF*, 505 bp)

Presence of the MazEF TA system

All 200 *E. faecalis* strains were analyzed by PCR, and findings were designated as positive if a distinct band was found at the expected size on an agarose gel. The PCR results showed that the *mazEF* TA system was found in all VRE isolates (79/79, 100%), however, only in 31.4% (n=38) of the 121 VSE isolates. This difference was statistically significant (p<0.001; 2-sided Fisher's exact test).

Discussion

VRE are increasingly found as responsible bacteria for a large number of nosocomial infections. VRE is known as a one of the challenging bacteria, which is able to pass its vancomycin-resistant gene to the methicillin-resistant *Staphylococcus aureus* (MRSA) [9]. Although there are many isolation of VRE in hospital settings, only a little information is available on the nature of plasmid-encoded vancomycin resistance gene. The mechanism of presence and retaining of mobile genetic elements is not understood yet.

The TA systems were found on the chromosome and plasmid of bacteria. Although the function of these TA systems is not fully elucidated yet, but some researchers suggest that it is responsible for stress tolerance [10], [11]. The TA loci were found in plasmid of many Gram negative bacteria, and best characterized in *Escherichia coli* [12]. The plasmid role of TA loci is in post-segregation killing that by disruption of antitoxin can cause suicide in bacteria [13].

The *mazEF* TA system was first found in the chromosome of *E. coli* [13]. The *mazE* is antitoxin and *mazF* is toxin and stable. The *mazE* is degraded with ClpA protease; therefore, in the absence of the genes encoding *mazEF*, the *mazE* will be degraded and MazF can kill the cell. The MazF toxin is an endoribonuclease, which is specific for ACA sequences [14], [15].

Our results demonstrate that the *mazEF* was positive in all VRE isolates (100%), but less prevalent (38/121, 31.4%) among the 121 VSE strains. The analysis showed resistance to vancomycin possibility harbored by plasmid containing TA loci. However, our analysis showed high prevalence of VRE also *mazEF* in strains isolated in Milad hospital. Interestingly, *mazEF* only were found in VSE, which collected in Milad hospital. All the VRE were positive for *mazEF* but in 31.4% of VSE were positive that may associate with the others antibiotics that not studied in current research. When the TA system contains on plasmid during binary fission, the results will be a survived daughter cells that inherit the plasmid. The daughter cell that does not inherit the plasmid harboring TA system will be killed. Because of the degradation of antitoxin, the stable toxin kills the cell. This is called "post-segregational killing" (PSK) [16]. By this way, bacteria that contain the TA loci on plasmid (commonly these plasmids also harbored the antibiotic resistance genes) will be survived and the bacteria without plasmid containing TA loci and so do not have the antibiotic resistant genes will be died. Our results suggested by activation of toxin (*mazF*) in VRE strain, it can be interesting target for antimicrobial therapy.

Conclusion

Our study demonstrated a positive relationship between the presence of vancomycin resistance and *mazEF* TA

system. This observation may introduce therapeutic options against a novel antimicrobial target in enterococci.

Notes

Competing interests

The authors declare that they have no competing interests.

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Please cite as

Sadeghifard N, Soheili S, Sekawi Z, Ghafourian S. Is the mazEF toxin-antitoxin system responsible for vancomycin resistance in clinical isolates of *Enterococcus faecalis*? *GMS Hyg Infect Control.* 2014;9(1):Doc05.
DOI: 10.3205/dgkh000225, URN: urn:nbn:de:0183-dgkh0002251

This article is freely available from

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Published: 2014-03-07

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