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A RETROSPECTIVE NEXT GENERATION SEQUENCING-BASED VIRULOME ANALYSIS OF THE FIRST VANA-TYPE ENTEROCOCCUS FAECALIS CLINICAL ISOLATE FROM BULGARIA

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A Retrospective next Generation Sequencing-based Virulome Analysis of the First VanA-type Enterococcus faecalis Clinical Isolate from Bulgaria

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In this study, we report the results from a retrospective next generation sequencing-based virulome analysis of the first VanA-type Enterococcus faecalis clinical isolate from Bulgaria. This strain was obtained from an 84-year-old female patient with clinical symptoms of urinary tract infection that started one week before the date of hospitalization. The vanA gene and its genetic environment are already identified; thus the whole-genome sequencing approach presented here is focused on the detection of the genes that contribute to the virulence of this isolate.

Keywords: *Enterococcus faecalis, virulome, vancomycin resistance, whole-genome sequencing, urinary tract infection*

Болгарияда анықталған VanA типті (Enterococcus faecalis) бірінші клиникалық изолятының жаңа ұрпақ секвенциясы негізінде вирусомалардың ретроспективті талдауы

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Бұл зерттеуде біз Болгарияда анықталған VanA типті Enterococcus faecalis бірінші клиникалық изолятының жаңа ұрпақ секвенциясы негізінде вируломаның ретроспективті талдауының нәтижелері жәнінде мәлімдейміз. Бұл штамм қабылдаудан бір апта бұрын басталған зәр шығару жолдарының инфекциясының клиникалық белгілері бар 84 жастағы науқас әйелден алынған. VanA гені және оның генетикалық ортасы қазірдің өзінде анықталған; осылайша, мұнда ұсынылған геномды секвенирлеудің бүкіл тәсілі осы изоляттың вируленттілігіне ықпал ететін гендерді ашуға бағытталған.

Негізгі сөздер: *Enterococcus faecalis, вирусома, ванкомицинге төзімділік, тұтас геномды секвенирлеу, зәр шығару жолдарының инфекциялары*

Ретроспективный анализ вирулома на основе секвенирования нового поколения первого клинического изолята Enterococcus faecalis типа VanA из Болгарии

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В этом исследовании мы сообщаем о результатах ретроспективного анализа вирулома на основе секвенирования нового поколения первого клинического изолята Enterococcus faecalis типа VanA из Болгарии. Этот штамм был получен от 84-летней пациентки с клиническими симптомами инфекции мочевыводящих путей, начавшейся за неделю до госпитализации. Ген VanA и его генетическое окружение уже идентифицированы.

Таким образом, представленный здесь подход к секвенированию всего генома направлен на обнаружение генов, которые способствуют вирулентности этого изолята.

Ключевые слова: *Enterococcus faecalis, вирулом, устойчивость к ванкомицину, полногеномное секвенирование, инфекции мочевыводящих путей*

Introduction

Enterococcus faecalis is a versatile lactic acid bacterium with a close relationship to human health and disease. This common commensal microorganism is among the earliest colonizers of the newborns' gastrointestinal tract and can be found as part of the normal intestinal flora in up to 90–95% of all people [1]. On the other hand, E. faecalis is also associated with severe nosocomial infections and multidrug resistance. In this aspect, the biggest threat for medicine and public health is the vancomycin-resistant clinical isolates (VREfs). They have been associated with limited therapeutic options and high mortality among infected individuals [2]. Over the last two decades, the incidence of nosocomial VREfs isolates in Europe and the USA was low [3].

In Bulgaria, only single VREfs isolates have been found so far, according to the annual reports of the European Antimicrobial Resistance Surveillance Network (http://atlas.ecdc.europa.eu). In contrast, recent studies have demonstrated multiple occurrences of vanA E. faecium strains in different Bulgarian hospitals for the period 2013–2017 [4]. To date, only one clinical VanA-type VREfs isolate from a Bulgarian hospital was subject to genetic characterization and the corresponding report was entirely focused on the structure and the localization of the vanA cluster [5]. The aim of our study was to perform a retrospective next-generation sequencing virulome analysis of this clinical isolate in order to better understand the molecular basics of its virulence.

Methods

Clinical case presentation

This study is focused on the clinical isolate E. faecalis BG475 that originates from an 84-year-old female patient with clinical symptoms of urinary tract infection that started one week before the date of hospitalization. The patient was diagnosed with chronic kidney failure and had been admitted to the Department of Nephrology of the Military Medical Academy (MMA) in Sofia, Bulgaria. She had no history of recent hospitalization. The VREfs isolate described here was obtained in December 2015 from a urine sample (>105 CFU/ mL) one day after the patient's hospital admission. Antimicrobial therapy consisting of intravenous imipenem (2x500 mg per day) was initiated.

Identification of the isolate

Species identification of the isolate was done by analyzing the assembled draft genome sequence using the Microbial Genomes Atlas (MiGA) Web server (http://microbial-genomes.org/). The included workflow for the NCBI Genome Database, Prokaryotic section was followed with default settings.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of the presented here E. faecalis BG475 isolate was determined by the minimum inhibitory concentration (MIC) gradient test (MIC Test Strip; Liofilchem, Roseto degli Abruzzi, Italy) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (http://www.eucast.org) for 2019.

Whole-genome sequencing and draft genome assembly

The whole genome sequencing of E. faecalis BG475 was performed on an Illumina HiSeq system (Illumina Inc., San Diego, CA) using 2x150-bp paired-end sequencing (BGI Group, Hong Kong, China). The resulting raw read pairs were then submitted to the online platform Galaxy (https://usegalaxy.eu/) where all subsequent data processing steps took place. Default parameters were used for all following software tools unless otherwise specified. The entire genome assembly procedure was performed using the following tools: FastQC [7] "id":"ITEM-1","issued":{"date-parts":[["0"]]},"title":"FastQC A Quality Control tool for High Throughput Sequence Da ta","type":"webpage"},"uris":["http://www.mendeley.com/documents/?uuid=8ecd0ca6-1f16-3f20-935c-7322eadb85c8"]}],"mendeley":{"formattedCitation":"[10]","plainTextFormattedCitation":"[10]","previouslyFormattedCitation":"[10]"},"properties":{"noteIndex":0},"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}, Trimmomatic [8] we could not find any tool or combination of tools that met our requirements in terms of flexibility, correct handling of paired-end data and high performance. We have developed Trimmomatic as a more flexible and efficient preprocessing tool, which could correctly handle paired-end data. Results: The value of NGS read preprocessing is demonstrated for both reference-based and reference-free tasks. Trimmomatic is shown to produce output that is at least competitive with, and in many cases superior to, that produced by other tools, in all scenarios tested. Availability and implementation: Trimmomatic is licensed under GPL V3. It is cross-platform (Java 1.5+ required, and SPAdes [9]. QUAST was used to calculate the draft genome's metrics [10].

Virulome screening

The virulome screening was performed using the

VFanalyzer tool available at the Virulence Factor Database (http://www.mgc.ac.cn/VFs/main.htm) [11].

Results

The antimicrobial susceptibility testing pointed out high-level resistance to vancomycin (MIC >256 mg/L), teicoplanin (MIC = 24 mg/L) and gentamicin (MIC > 1024 mg/L), resistance to levofloxacin (MIC > 32 mg/L) and susceptibility to ampicillin (MIC = 0.25 mg/L), imipenem (MIC = 0.25 mg/L), linezolid (MIC = 1 mg/L), nitrofurantoin (MIC = 3 mg/L) and tigecycline (MIC = 0.064 mg/L).

Based on these results, the patient received an antimicrobial therapy consisting of intravenous imipenem 500 mg twice per day. As a consequence, the patient's clinical symptoms diminished within the next 36 h. The 2-week follow-up culture was negative for E. faecalis.

The whole-genome sequencing generated 4,400,398 raw read pairs. They were next uploaded to the Galaxy online platform where the 3.38-Mb draft genome assembly of E. faecalis BG475 was generated. It was comprised by 83 contigs larger than 1000 bp (largest contig – 602,910 bp) with an N50 value of 158,001 and an average GC content of 37.01%.

The initial identification of the BG475 isolate as E. faecalis was confirmed by analyzing the assembled draft genome sequence. Its virulome was found to be composed of many genetic determinants, including virulence factors involved in adherence (aggregation substance, collagen adhesin, Epb pili, EcbA, and Efa), antiphagocytosis (capsule-production related cps operon, including all 7 open reading frames that are essential for this process), biofilm formation (BopD transcriptional regulator and Fsr locus), exocellular enzymes (gelatinase, hyaluronidase, and serine protease), and toxins (cytolysin) (Table 1).

Discussion

BG475 is the first vancomycin-resistant E. faecalis clinical isolate found in Bulgaria. Previous study demonstrated that it harboured a vanA gene cluster and its genetic structure was determined [5]. Nevertheless, the set of genes that contributed to the virulence of this strain has not been investigated so far. The retrospective next generation sequencing-based virulome analysis of the BG475 isolate, presented in the current study, revealed genetic determinants that belong to five different classes. Some of their products have already been associated with urinary tract infections. One example is the aggregation substance (AS) which was found to mediate adhesion of E. faecalis to a variety of eukaryotic cells in vitro, including renal tubular cells [12]. Additionally, the ASA protein has the ability to protect the bacterial cell from being killed by polymorphonuclear leukocytes, that are an important urinary tract defense element. Nevertheless, it is worth mentioning that this gene product is not recognized as a major contributor to urinary tract colonization in a mouse model-based study [13]. Another adherence-associated virulence factor, the EbpA pilin, is also known to play a role in catheter-associated urinary tract infection models. [14].

Recent study revealed various genotype-phenotype correlations of key virulence factors in clinical E. faecalis isolates causing urinary tract infections [15]. Their findings confirmed that the urinary tract pathogens possess strong biofilm forming ability since the biofilm formation enhances the crucial step of adherence, which protects the bacteria from being flushed by urine flow. Biofilm strength was found to be correlated with the presence of the sprE and gelE genes and both of them were identified in the draft genome sequence of E. faecalis BG475. Moreover, our isolate was also found to harbor all three genes in the fsr locus (fsrA, fsrB and fsrC) that are required for the expression of gelE. Their presence serves as a sufficient biomarker for gelatinase activity in E. faecalis isolates obtained from the urinary tract [15]. Finally, the cyl genes identified in our virulome screening were also recognized as a biomarker for hemolytic activity, which was associated with increasing severity of the infection [15].

Taken together, all these findings suggest that the genetic profile of E. faecalis BG475 highlights it as a potent urinary tract pathogen. In combination with the vanA gene cluster, this isolate becomes a serious threat to all immunocompromised patients in Bulgarian hospitals. Infectious agents of this type represent a significant source of morbidity and mortality, and whole-genome sequencing is a very promising approach for comprehensive systems-

Table 1. Virulence genes identified in the E. faecalis BG475 isolate.

Virulence factors class	Virulence factors	Identified genes
Adherence	Aggregation substance (AS)	*asa1, EF0149*
	Collagen adhesin (ace)	*ace*
	Ebp pili	*ebpA, ebpB, ebpC, srtC*
	EcbA	*ecbA (EF1896, EF2347)*
	EfaA	*efaA*
Antiphagocytosis	Capsule	*cpsA/uppS, cpsB/cdsA, cpsC, cpsD, cpsE, cpsF, cpsG, cpsH, cpsI, cpsJ, cpsK*
Biofilm formation	BopD transcriptional regulator	*bopD*
	Fsr locus	*fsrA, fsrB, fsrC*
Exocellular enzymes	Gelatinase	*gelE*
	Hyaluronidase	*EF0818*
	Serine protease	*sprE*
Toxins	Cytolysin	*cylL-l, cylL-s, cylM, cylR2*

level analysis of their virulence potential. Nonetheless, the draft genome-based virulome analysis described here also has a few limitations. They lie mainly in the poorly understood relations between many sequence variants that can be found in the coding sequences/promotor regions of virulence-related genes and their potential effect on the expression and/or the functionality of the encoded proteins. A possible way to overcome these restrictions will be the application of additional -omics technologies (like transcriptomics) and integration of their results with the obtained genome sequence.

Nucleotide sequences

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAHZNN000000000. The version described in this paper is version JAHZNN010000000.

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Competing interests

None declared.

Ethical approval

Not required.

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