Department of Clinical Microbiology, Rigshospitalet
Copenhagen, Denmark

P0815

Inducible aminoglycoside resistance in vancomycin resistant *Enterococcus faecium*

AUTHOR LIST
Marie Juhl, Rikke Overgaard Kiilerich, Stine Radmer, Karen Leth Nielsen, Frederik Boëtius Hertz, Niels Frimodt-Møller
AFFILIATION
Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark

INTRODUCTION

Vancomycin resistant *Enterococcus faecium* (VRE) are responsible for hospital outbreaks around the world. High-level aminoglycoside resistance is common in these strains. The most prevalent resistance mechanism is inactivation by enzymatic modification where genes encode a transferase that adds a functional group to the aminoglycoside. Some VRE can contain resistance genes without conferring resistance, one reason for this is pseudogenes.
The aim of this project is to examine whether inactive aminoglycoside resistance genes can be activated and thereby cause resistance.

CONCLUSION:

The aminoglycoside resistance can be induced when the *E. faecium* strains are exposed to an aminoglycoside, but the two VRE strains with similar aminoglycoside-resistance genes varied in their response to induction with gentamicin, tobramycin or amikacin. Further studies are needed to investigate the genetic background for the observed induction of resistance.

METHOD

Two clinical VRE strains containing the bifunctional gene, *aac(6')-Ie-aph(2")-Ia*, without conferring phenotypic resistance to gentamicin, tobramycin, or amikacin and containing *aph(3')-III* without giving resistance to amikacin were studied (Table 1).
To examine if the resistance can be induced, the strains were exposed to each of the three aminoglycosides at ½ and ¼ of the MIC and grown for 72-hours, replacing the media with the same concentrations every 24-hours. Hereafter, the bacteria were spread on agar plates containing the MIC for each aminoglycoside.

		Aminoglycoside-resistance genes (% coverage) [a]				Aminoglycoside MICs (mg/L)			
No.	MLST	*aac(6')-Ii*	*ant(6')-Ia*	*aac(6')-Ie-aph(2")-Ia*	*aph(3')-III*	Streptomycin	Gentamicin	Tobramycin	Amikacin
20	117	100.00	100.00	91.04	100.00	>512	**6**	**32**	**32**
22	117	100.00	100.00	91.04	100.00	>512	**6**	**24**	**24**

Table 1, *E. faecium* isolates with mismatch between genotype and phenotype. The table shows gene coverage from ResFinder BLAST, the MIC values determined by Etest and the MLST types. Colours indicate which genes encode for resistance with both *aac(6')-Ie-aph(2")-Ia* and *aph(3')-III* conferring resistance to amikacin. Underlining numbers indicate mismatch between genotype and phenotype
[a]Nucleotide identity was >99,5% for all gene identification.
Reference: JAC, 2021, 76: 2215-2217

To confirm the resistance disk diffusion test and Etests were performed on the colonies from the agar plates containing the aminoglycosides. The resistant colonies were afterwards grown for three days without antibiotics to confirm the stability of the resistance.
The genomic structure will be analysed with Nanopore sequencing.

Changes before and after exposure to amikacin in isolate 20				
	Aminoglycoside MICs (mg/L)			
	Gentamicin	**Tobramycin**	**Amikacin**	
Phenotype before	6	32	32	
Phenotype after	16	>1024	>256	
	Aminoglycoside-resistance genes (%coverage / identity)			
	aac(6')-Ii	***ant(6')-Ia***	***aph(3')-III***	***aac(6')-Ie-aph(2")-Ia***
Genotype before	100 / 99.64	100 / 100	100 / 99.87	91.04 / 100
Genotype after	100 / 99.64	100 / 100	100 / 100	91.04 / 100

Table 2, phenotypic and genotypic changes for isolate 20 after exposure to amikacin and an amikacin disk. Highlighted in red indicate the changes between before and after exposure.

RESULTS FOR ISOLATE 20

- Exposure to amikacin for three days and tested by an amikacin disk showed colonies growing inside the inhibition zone.
- These colonies exhibited high level resistance towards tobramycin (>1024 mg/L) and amikacin (>256 mg/L) but not gentamicin (16 mg/L) (Table 2).
- The only change in the aminoglycoside-resistance genes was a silent mutation in *aph(3')-III* (Table 2).
- A core genome SNP analysis was conducted and found two missense mutations leading to amino acid changes in a heat-inducible transcriptional repressor HrcA and an alpha-hydroxy-acid oxidizing protein (Table 3).
- When testing the stability of the induced resistance the MIC did not change.
- Gentamicin and tobramycin did not induce resistance in this isolate.

#Chrom	Position	Reference	Alt	Mutation type		Amino acid change		Type	Gene
NC_017960	1633468	G	T	Missense variant	Protein coding	Asp8Glu	Intragenic variant	SNP	Heat-inducible transcriptional repressor HrcA
NC_017960	2065222	G	A	Missense variant	Protein coding	Pro194Leu	Intragenic variant	SNP	Alpha-hydroxy-acid oxidizing protein

Table 3, SNP analysis on the genome before and after exposure of amikacin. Table showing a reference genome and information about the changes in the two genes.

RESULTS FOR ISOLATE 22

- High level resistance towards gentamicin (>1024 mg/L), tobramycin (>1024 mg/L) and amikacin (>256 mg/L) after exposure to gentamicin (½ MIC for two days)
- The MIC did not change when the stability was tested.
- Amikacin and tobramycin did not induce resistance.
- The genetic structure is under further investigation by Nanopore sequencing to elucidate the genomic structure.

Correspondence:
Department of Clinical Microbiology, KMA, Rigshospitalet
Ole Maaløes vej 26
2200 Copenhagen N
Denmark
Email: Marie.Juhl.01@regionh.dk

24th of April 2022