Introduction
An increase in antifungal-resistant Candida strains has been reported in recent years. Next Generation Sequencing (NGS) allows the investigation of genetic variations in different genes in large populations. The aim of this study was to detect mutations in resistance genes using NGS technology and to determine the impact of the underlying mechanisms of azole and echinocandin resistance.

Resistance Testing – EUCAST reference method: anidulafungin, micafungin, caspofungin, fluconazole, posaconazole, voriconazole,itraconazole and isavuconazole

Next Generation Sequencing with Illumina MiSeq – Targeted Resequencing of
- ERG11, ERG3, TAC1 and GSC1 in C. albicans
- ERG11, CgPDR1, FKS1 and FKS2 in C. glabrata

Bioinformatic Analysis
- FastQC, Trimmomatic, Assembly Bowtie2, Variant Calling – VarScan and SnpEff

Results
140 different mutations
- 87 silent mutations
- 53 missense mutations
  - 42 mutations found to be presumably causal
  - 13 of these are reported for the first time

C. albicans
ERG11: 7 point mutations in azole-resistant strains
TAC1: 5 potential gain-of-function mutations in the transcription factor
ERG3: 5 potential loss-of-function mutations and two homozygous premature stop codons
GSC1: 5 target mutations in all echinocandin-resistant strains

C. glabrata
ERG11: No mutations were found in azole-resistant C. glabrata
CgPDR1: In 10 out of 13 azole-resistant isolates
  - 11 different potential gain-of-function mutations in the transcription factor CgPDR1
FKS: target mutations in all echinocandin-resistant strains

Conclusion
All echinocandin-resistant Candida strains with a MIC at least two titers above the clinical breakpoint showed a mutation in the hotspot regions of FKS1/2 or GSC1

Usually a cross resistance in echinocandins → one C. glabrata strain (P667T in FKS2 – HS1) showed an isolated susceptibility to micafungin despite high anidulafungin and caspofungin MICs → Anidulafungin does not always predict resistance to other echinocandins

azole-resistant C. glabrata showed no ERG11 mutations → CgPDR1 seems to be a more important factor

Relevance of TAC1 in C. albicans remains unclear

Loss-of-Function mutations in ERG3 - two homozygous premature stop codons
high rate of variances → differentiation of polymorphisms and causal mutations is very important

Not all multi-resistant strains showed mutations which explain echinocandin and azole resistance → alternative mechanisms?

Next Generation Sequencing is an excellent method to detect resistance mutations in many potential resistance genes in a large population.