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Detection of azole susceptibility patterns in clinical yeast strains isolated from 1998 to 2008

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SUMMARY

4,860 clinical yeast isolates (25 genera, 47 species) were tested in parallel to fluconazole, itraconazole, ketoconazole, and voriconazole. After re-evaluation of all species according to their current valid taxonomic denominations, the range of the top four of the dermatology, gynaecology and paediatrics associated species from superficial infections was similar to those isolated from other wards with mainly systemic/invasive infections. *Candida albicans* (44.7%) was the most frequent pathogen followed by *C. glabrata, C. tropicalis*, and *C. parapsilosis*. The MIC-assessment revealed for the ten-year test period an overall azole-susceptibility of about 75%, and ~80% for their associated ICUs. The overall susceptibility of the isolates from systemic and superficial infections to the four azoles was 79% and 80% respectively, and demonstrates a high *in vitro* activity. When two test periods (1998-2001 and 2002-2008) were compared by characteristic MIC values and multi-azole resistance, no significant increase could be detected in azole susceptibility/resistance over the two periods, respectively, over the total investigation period of ten years. This holds true when the characteristic MIC values were compared with those from different azole susceptibility studies from similar time periods and from different investigators around the world (1991 to 2010). With a new method, susceptibility pattern analysis for fungi, detailed information of multi-resistant microorganism populations could be obtained, and different characteristic resistance patterns in clinical yeast species detected. Although at a relatively low level, multi-resistance was seen in individual species populations demonstrating resistance to two (6.7%), three (4.4%), or all four (4%) azoles tested. A level of 4% and 2% fourfold parallel resistance was also determined in *Candia* spp. and non-*Candida* spp. derived of blood culture isolates.

KEY WORDS: Susceptibility pattern analysis, Azole multi-resistance, Yeasts, Dermatology, Gynaecology, Paediatrics.

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INTRODUCTION

Yeast infections continue to represent a major health care threat in dermatology, gynaecology, and paediatrics (DGP). In dermatology, dermatophytes are still the prevailing type of fungi causing infections of the skin and nails (Goldstein and Goldstein, 2013), however, yeasts are the second most important cause of nail fungal infections in the world. In this study, *Candida albicans* and *Candida parapsilosis* were the two most common species for this infection type. In addition, *Candida* species with resistance to azoles causing superficial and local mucosal infections are additionally responsible for a high medico-social burden in gynaecology and paediatrics (Manzano-Gayosso *et al.*, 2010). Such infections, like thrush, generally affect gastroin-

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testinal, vaginal, oesophageal, and oral pharyngeal mucosa. Repeated recurrences are known (Sobel, 1997), e.g. as recurrent vulvovaginal candidiasis (RVVC). Oral-pharyngeal candidiasis is common among HIV-infected patients and is considered to be an important marker for the onset of AIDS (Kabir and Ahmad, 2013). *Candida* and not-*Candida* (NCY) yeast species are also involved in chronic mucocutaneous candidiasis (CMC) (Kabir and Ahmad, 2013; Miceli *et al.*, 2011; Arendrup *et al.*, 2008; Zilberberg *et al.*, 2008; Eyrich *et al.*, 2010), and occur in patients with atopic dermatitidis (AD) (Zincevicience *et al.*, 2011). CMC defines a heterogeneous group of orphan and inherited syndromes characterised by chronic and recurrent infections of the skin and mucosa with the yeast *Candida* (Akdis *et al.*, 2006), whilst AD is a multifactorial disease comprising both hereditary and environmental factors. In these entities, the three genera *Candida*, *Malassezia* and *Rhodotorula* play an essential role (Zincevicience *et al.*, 2011). Up to 75% of healthy individuals carry the yeast *Candida* as part of their oral microbiota.

In the past, there have been important changes in the epidemiology of invasive candidiasis with an overall shift toward non-*Candida albicans Candida* (NCAC) species, particularly *Candida glabrata*. In addition, previously uncommon pathogens such as *Trichosporon* spp. and *Geotrichum* species, with reduced susceptibility to antifungal agents have emerged and infections by opportunistic yeasts are on the rise (Miceli *et al.*, 2011). Since resistance to antimycotic drugs is reported to evolve, species identification and antifungal susceptibility testing has also become essential in DGP-clinical settings (Schmalreck *et al.*, 2012a). NCAC isolates are generally more resistant to azoles than *C. albicans* strains (Meunier, 1989; Candido *et al.*, 1998; Otero *et al.*, 1999; Morace *et al.*, 1991; Arendrup *et al.*, 2008; Zilberberg *et al.*, 2008; Eyrich *et al.*, 2010; Miceli *et al.*, 2011; Silva *et al.*, 2013). Particularly in patients with VVC, several authors have found an increased prevalence of *C. glabrata* due to a selection mechanism related to the frequent use of imidazoles (ketoconazole and miconazole) (Spinillo *et al.*, 1994; Richter *et al.*, 2005; Gross *et al.*, 2007). VVC caused by *C. albicans* strains, resistant to fluconazole without and with previous azole treatment, has also been reported (Shahum *et al.*, 2007; Mulu *et al.*, 2013). It has been demonstrated that specimens from superficial and non-dermatophyte infections in DGP-wards and their related ICUs share common yeast pathogens.

The present study re-evaluated and compared the MICs, respectively the resistance data from the multicentre studies (MCS) (Czaika *et al.*, 2013; Schmalreck *et al.*, 2012a; Schmalreck *et al.*, 2014a), dealing with superficial (SFI) and systemic infections (SYS) partly using newly introduced methodologies for:

- Differences in species profiles of isolates from superficial infections in comparison to species of isolates from other clinic specialities dealing with systemic yeast infections;
- Changes in MIC-profiles when the "new" and currently valid nomenclature for yeast species is applied (see "Panel" for the current valid names);
- Detection of changes in azole MIC-resistance patterns, respectively, of possible parallel (cross) resistance to fluconazole (FLC), itraconazole (ITR), ketoconazole (KET), and voriconazole (VOR) by susceptibility pattern analysis (SPA);
- Evaluation of potential susceptibility trends for the investigation period of 10 years.

MATERIAL AND METHODS

Isolates and identification

4,860 clinical yeast isolates from Germany/Austria were tested in parallel during several MCS between 1997 and 2009, for their susceptibility to fluconazole (FLC), itraconazole (ITR), ketoconazole (KET), and voriconazole. Of the isolates, derived from the German University hospitals in Berlin (Charité), Dresden, Leipzig, Münster, and Munich, 1,448 strains (29.8%) were derived from dermatology (DER), gynaecology (GYN), and paediatrics (PED), 1,063 (21.8%) from clinics/wards with mainly systemic infections (SYS), 1,082 strains (22.3%) from general medicine (GM), 493 (10.1%) from other clinic specialities dealing with invasive infections (INV) of lower risk patients, 139 (2.9%) from DGP-associated, and 635 isolates (13.1%) from all other intensive care units (ICUs). Iden-

tification and differentiation of the isolates and the epidemiology of SFI-related (Czaika *et al.*, 2013), and from SYS-related species have been described (Schmalreck *et al.*, 2012a; Schmalreck *et al.,* 2014a).

Nomenclature

According to the "one fungus one name" principle, effective since 2013 (Hawksworth *et al.*, 2011; McNeill *et al.*, 2011), all species have been re-evaluated/renamed according to their current valid names (Schmalreck *et al.*, 2014a) as published in SpeciesFungorum (Species Fungorum, 2014), respectively in MycoBank (MycoBank, 2014). These "new" taxonomic denominations were applied to all species throughout this paper (see Panel).

Susceptibility testing

All antifungal susceptibility testings were performed by the validated microdilution method according to DIN 58940-84 (DIN 58940-84, 2002) with pre-manufactured log_2-dilution rows in 96-well microdilution trays (Merlin GmbH, Germany) with the antifungal agents (AFAs): fluconazole (FLC; test range: 0.0625-128 mg/l), itraconazole (ITR: 0.008-16 mg/l), ketoconazole (KET: 0.016-16 mg/l), and voriconazole (VOR: 0.008-16 mg/l). FLC and VOR were provided by Pfizer GmbH Germany free of charge. Itraconazole and ketoconazole, and RPMI medium were purchased from Sigma GmbH (Munich, Germany). DIN (yeast) susceptibility test (YST) medium (DIN 58940-84, 2002) was manufactured by Sifin GmbH (Germany) and produced in (sterile) liquid form by heipha GmbH (Germany). All materials were provided as single batches together with the microdilution trays as "ready to use" to the test centres. The endpoint determinations (MIC) were performed after 24 h incubation at 36°C±1°C. When tested against slow growing species this may have been done 48h after the respective appropriate growth times. In all instances a second reading was performed 24h after the first one and documented. All MIC values were read visually as the lowest concentration of the AFA that caused no growth or at least a significant reduction of the growth (≥80%) relative to that of the growth control in the microdilution trays. Aside from *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019, another 25 quality control (QC) strains, e.g. as recommended by DIN (DIN 58940-84, 2002), CLSI (CLSI, 2012), EUCAST (EUCAST, 2013), and well characterised, PCR and FT-IR typed clinical isolated were tested in parallel (data on file). As there were no (clinical) breakpoints (BPs) from EUCAST (EUCAST, 2013) for itraconazole and ketoconazole, and only a few for fluconazole and voriconazole and some *Candida* species available, the breakpoints applicable to assess the MICs into susceptible (S), intermediate (I), and resistant (R), were appropriate to the DIN microdilution susceptibility testing method (DIN 58940-84, 2002; DIN-Fachbericht, 2007) and applied for the comparison purposes here as follows: fluconazole, S: ≤2, R: > 8; except for *C. albicans*, *C parapsilosis*, *C. tropicalis*, *Meyerozyma guilliermondii*, FLC, S: ≤2, R: >4; *C. glabrata*, S: ≤4, R: >8; ITR, S ≤0.125, R: >0.5; KET, S: ≤0.25, R: >0.5, except for *C. albicans*, *C parapsilosis*, and *C. tropicalis*, S: ≤0.125, R: >0.125; VOR, S: ≤1, R: >2, except for *C. albicans*, *C parapsilosis*, and *C. tropicalis* S: ≤0.125, R: >0.125, for *C. glabrata* and *I. orientalis* S: ≤0.5, R: >1. Thus, at that time of re-evaluation the chosen breakpoints were close to those currently issued by EUCAST (2013).

In addition, instead of the MIC assessment into susceptible (S), intermediate (I) and resistant (R) as described by CLSI (CLSI, 2008) and EUCAST (EUCAST, 2012), called in this manuscript as "3-leg system", the "2-leg" system according to Grimm (1991) with only S* and R* was performed in addition. Where appropriate, this was performed to achieve epidemiologically, respectively microbiologically balanced MIC comparisons, and to avoid MIC assessments without "S" or "R", when the result is either only "IR" or "IS" or "I". Analogous to the bacterial evaluations (Grimm, 1991), and to cover the intermediate (CLSI: dose-dependent) tested isolates, which at repeated exposure to the drug (Van Minnebruggen *et al.*, 2010) or by mutations (Rathod *et al.*, 2012) may become resistant, the intermediate (I) strains are added in the two-leg system by 40% to the susceptible (S) fraction (S* = S + 40% I) and by 60% to the resistant (R) category (R* = R + 60% I; 100% = S* + R*). All the MICs displayed are derived from the antilog of the log_2-MIC-calculations. The epidemiological cut-off value (ECV) was

calculated according to Arendrup *et al*. (2009), with the median MIC as basis. When not otherwise indicated, all percentage values are given in round figures. All statistical analysis were performed with *SAS*® software (*SAS*® Institute, Cary USA; Heidelberg, Germany).
Multiple resistance (MR) was defined for an isolate, when two or more antifungal agents, independently of any substance class, tested resistant, *i.e.* representing a random susceptibility pattern. Parallel resistance (PR), in literature mostly referred to as "cross resistance", was defined here as resistance of a given isolate to all azoles (complete PR) or as resistance to more than one, but not to all substances of the azoles (partial PR). As all antifungal agents in this evaluation fall into the azole-class, the 2-fold, and 3-fold PR are classified as incomplete PR, and the 4-fold PR as complete PR.

Compilation of specimens and clinics/wards

To ease the assignment and comparisons of MICs and the patient-related factors, origin and type of specimens respectively, those data were appropriately merged and subsumed in large groups, e.g. as surgery (SURGERY: abdominal, aesthetic, general, heart, plastic, vascular, neurosurgery); general medicine (GM: angiology, bronchoscopy, dialysis, endocrinology, gastroenterology, internal medicine, emergency room, nephrology, pneumology, policlinics, rehabilitation centre, standard care, tropical medicine); external (EXTERN: doctor offices, external wards, outside laboratory); swabs (SWAB: surfaces, all body parts, skin, stoma, ear, bone); aspirates (ASPIRATE: materials from punctures such as pleura, ascites, abscess, bursa, pericardial, pleura, drain fluid, bronchoscopy materials); catheters (CATHETER: indwelling, central, vascular, venereal, ports, catheter tips, anaesthetic tube, drains); liquids (FLUID: except blood and blood cultures and urine; sterile body fluids, bile, liquor, dialysates, BAL, pleura, lachrymal, synovial, serum, and jetting liquids e.g. from bronchia, sinus, stomach, bladders and renal pelvis, other secrets); solid material (Materials: except stool; tissue/lung tissue, bone-marrow, tumour/neoplasm, abscess, pus, spleen, bone, liver, biopsy materials); devices (DEVICE: contact lenses, artificial joints, dialysis access, haemodialysis grafts, cardiac devices such as heart valves, pacemakers, ICDs, VADs, penile implants); dermatological materials (MAT-D: skin, skin scrapings, nails/nail scrapings, plucked hair, dandruff, scales, wound material/secret); gynaecological materials (MAT-G: scrapings, genital-, prostate secrets, ejaculate, vaginal discharge, vaginal sponges, diaphragms, tampons, intrauterine devices, implants, alloplastic materials, and piercings); urine (non-sterile, mid-stream, punctuate, catheter); paediatric materials (MAT-P: skin biopsy, fibroblasts, dry blood, other solid or liquid materials derived from paediatric wards). To distinguish between the origin of the specimens from different body sites, each specimen (solid material/liquid/swab) was either categorized as "STERILE" or "NON-STERILE" (MAT-TYPE), and additionally grouped according to its texture, either as solid material (MAT-S), liquid material (including discharges or exudates; MAT-F), or as swab (SWAB).

Susceptibility pattern analysis

Susceptibility pattern analysis was adapted to the resistance pattern analysis (SPA) described by Fegeler *et al*. (1998). The susceptibility pattern (SP) obtained by susceptibility pattern analysis (SPA) was defined as the artificial array of all assessed MICs of the different parallel tested AMA, however for all evaluation purposes, in a pre-selected sequential arrangement called SP-basis, e.g., SP: FLC-ITR-VOR-KET, where according to the MIC assessment, for each strain a specific susceptibility pattern, e.g., SP: R_{FLC}-I_{ITR}-R_{KET}-S_{VOR} was assigned accordingly, and R (resistant) may be replaced by "S" (susceptible) or "I" (intermediate) or vice versa.

RESULTS AND DISCUSSION

Species distribution

In the context of 4 German/Austrian MCS from 1997 to 2009, 15,606 clinical yeast isolates were tested for their *in vitro* susceptibility against various antifungal agents (Czaika *et al*., 2014; DIN, 2007; Schmalreck *et al*., 2012a; Schmalreck *et al*., 2014a). Thereof, 4,860 strains (31%) were tested in parallel to FLC, ITR, KET, and

VOR. The species distribution per clinic speciality (CSP) is shown in Table 1. For investigation of possible epidemiological and azole-susceptibility trends the total time span from 1998 to 2008 was split into two test periods, P1 (1998-2001) and P2 (2002-2008). These two periods were chosen to obtain the most appropriate intersection in respect to isolate numbers, to the marketing of voriconazole in 2002 (splitting analogous to Guinea *et al*., 2008 for *Aspergillus* species), and to the increasing incidence, respectively, to the new rise in HIV infections in 2002 (Robert Koch Institute, 2011).

As there could be no significant species-specific differences detected by comparing both investigation periods P1 and P2, only the isolate distributions for total strains, all clinics/wards, and all ICUs are additionally given for both test periods in Table 1. The higher number of isolates tested in the second investigation period does not necessarily count as a trend to increasing species rates because due to the partially unevenly isolated low numbers of rare, infrequently isolated species. Therefore, although no collaborative studies took place in 2005, the overall raised isolate number (+248 strains / +16%) in P2 may be due to the different strain contributions of the participating institutions according to their bed capacity, and their laboratory throughput. In addition, it has to be taken into account that the species reported from the MCS are only a snapshot of the corresponding year and may be therefore inhomogeneous, respectively biased, because the MCS isolation periods (consecutive sampling up to a given number of strains) generally lasted only for three to four months at the beginning or towards the end of the year. Despite these shortcomings, the close sample sizes and small differences between the species populations from the testing periods P1 and P2, and the confirming statistics the available data may allow appropriate comparisons and MIC trend determinations.

It was shown recently that patients treated for their superficial infections in dermatology, gynaecology, and paediatrics (DGP) and their associated ICUs share the same most significant clinical yeast species worldwide (Czaika *et al*, 2014). If this parallels the distributions in CSPs dealing with invasive, systemic infections (SYS/INV), the isolates (N=4,860) included 1,448 (26.6%) DGP and 774 (15.9%) ICU (thereof 139 (2.9%) DGP-ICU isolates) strains. They were re-evaluated according to their FLC/ITR/KET/VOR *in vitro* susceptibilities for MIC profiles, and order and magnitude of azole multi-resistance. All samples were derived from 17 different (consolidated) clinics/wards and ICUs. In order to condense the sample origins and to ease further comparisons, these clinics/wards were further merged according to the prevailing infection type, severity of infection (Czaika, 2013); e.g. clinics with predominantly high-risk patients and systemic infections (SYS: 1063/21.9%), lower-risk patients and invasive infections (INV: 493/10.1%), mixed infections according to the aggregated specialities in general medicine (GM: 1,082/22.3%), and of ICUs (ICU: 635/13.1). From a total of 4,860 clinical isolates, 2,348 (48.3%) were derived from normally sterile (ST) body sites/devices, and 2,512 (51.7%) from non-sterile (NST) compartments. From liquids 2,650 (54.5%) strains were isolated, from solid or semisolid materials 803 (16.5%), and from swabs 1,407 (29%) clinical isolates. The five most prevalent species were almost identical in their isolation frequency for all clinical specialities, and the appropriate individual species distributions for the individual wards are given in Table 1. The most frequent *Candida* species were: *C. albicans* in the upper range of 38% (radiology and transplantation) to 78% (anaesthetics), and in the lower range of 24% (dermatology) to 33% (neurology); ICUs: 47% (ICU-G) to 92% (ICU-D); *C. glabrata*, 0%/3% (extern/eye) to 27% (transplantation), ICUs: 0% (ICU-D) to 24% (ICU-G); *C. tropicalis*, 0% (extern/eye) to 17% (dental), ICUs: 6% (ICU-G) to 13% (ICU-P); *C. parapsilosis*, 0% (anaesthetics) to 67% (eye), ICUs: 0% (ICU-D/ICU-G) to 4% (ICU-P); and *C. dubliniensis* from 0% (anaesthetics, dental, eye, gynaecology, orthopaedics, radiology) to 5% (HNO), and 1% in ICUs. Not listed here are non-*Candida* (NCS) species or species which were isolated only once, or due to a single outbreak within the 10-year investigation period (e.g., *C. africana*, *Prototheca* spp.), those isolated were not further considered which occurred only in one investigation period (e.g., *E. dermatitidis*, *M. furfur*). The five most frequent isolated NCS species were: *Issatchenkia orientalis,* 0% (wards: den-

tal, eye, urology) to 17% (ear, nose throat), ICUs: 5% (ICU-P to 12% (ICU-G); *Clavispora lusitaniae* 0% (dental, extern, eye, radiology), ICUs: 0% (ICU-D, ICU-P) to 12% (anaesthetics); *Kluyveromyces marxianus* 0% (anaesthetics, dental, extern, radiology), to 5% (neurology), ICUs: 0% (ICU-D, ICU-G) to 1% (other ICUs); *Saccharomyces cerevisiae* 0% (extern, eye, neurology, orthopaedics) to 9% (radiology), ICUs: 0% (ICU-D, ICU-G) to 2% (ICU-P); and *Meyerozyma guilliermondii* 0% (anaesthetics, dental, extern, eye, ear, nose, throat, neurology, radiology) to 4% (dermatology), ICUs: 0% (ICU-D, ICU-G) to 2% (ICU-P). Additionally, it can be

TABLE 1 - *Number of species (n >10), specimens derived predominantly from sterile (ST) and non-sterile (NST) body sites, and main specimen types (MAT-ART) together with their percentage of distribution (%) per clinic speciality/ward. For total isolates, total wards, and total ICUs*

Clinic speciality/Ward	*Frequency (total isolates: N=4,860)*	*% of total i(N)*	*Candida albicans (n=2,173)*	*Candida glabrata (n=889)*	*Candida tropicalis (n=437)*	*Candida parapsilosis complex (n=293)*	*Candida dubliniensis (n=46)*	*Candida sake (n=22)*	*Candida inconspicua (n=19)*	*Issatchenkia orientalis (n=328)*	*Clavispora lusitaniae (n=82)*	*Kluyveromyces marxianus (n=70)*
Total isolates[1]	**4,860**	**100.0**	**44.7**	**18.3**	**9.0**	**6.0**	**1.0**	**0.5**	**0.4**	**6.8**	**1.7**	**1.4**
All isolates in P1	1937	39.9	42.0	17.5	8.4	6.8	0.6	0.5	0.6	5.3	2.1	1.0
All isolates in P2	2923	60.1	46.5	18.8	9.4	5.5	1.2	0.4	0.3	7.7	1.4	1.7
All clinics and P1[2]	1855	38.2	40.6	18.0	8.5	7.0	0.7	0.5	0.6	5.2	1.2	1.0
All clinics and P2[2]	2231	45.9	44.9	17.9	9.3	6.2	1.3	0.5	0.3	8.2	1.4	1.9
All ICUs and P1[3]	82	1.7	73.2	6.1	6.1	2.5	0.0	0.0	0.0	7.3	0.0	1.2
All ICUs and P2[3]	692	14.2	51.7	21.7	9.8	3.3	0.9	0.4	0.1	6.2	1.6	1.2
Anaesthetics	32	0.7	**78**	**4**	**6**					**6**	**6**	
Dental	23	0.5	**39**	**22**	**17**	**18**		**4**				
Dermatology	356	7.3	**24**	**10**	**2**	**10**	**2**	**1**		**4**	**1**	**1**
External	39	0.8	**41**	**3**		**9**	**3**			**3**		
Eye	21	0.4	**29**			**67**						
General Medicine	1082	22.3	**45**	**20**	**9**	**7**	**1**	**1**	**1**	**7**	**2**	**2**
Gynaecology	519	10.7	**53**	**18**	**4**	**3**			**1**	**7**	**1**	**2**
Neurology	78	1.6	**33**	**21**	**15**	**4**	**1**			**12**	**1**	**5**
Oncology	236	4.9	**45**	**17**	**3**	**8**	**0.4**		**2**	**11**	**2**	**1**
Orthopaedics	49	1.0	**43**	**23**	**16**	**6**				**2**	**2**	**2**
Paediatrics	573	11.8	**44**	**14**	**13**	**6**	**1**	**1**	**0.2**	**8**	**3**	**1**
Radiology	34	0.7	**38**	**15**	**12**	**6**				**15**		
Surgery	443	9.1	**42**	**21**	**13**	**5**	**2**	**1**		**7**	**1**	**1**
Transplantation[4]	269	5.5	**38**	**27**	**14**	**2**	**1**			**6**	**2**	**2**
T-N-E4	144	3.0	**41**	**13**	**10**	**8**		**5**	**1**	**17**	**1**	**3**
Urology	188	3.9	**47**	**23**	**9**	**8**		**1**	**1**		**3**	**2**
ICU[5]	635	13.1	**52**	**21**	**9**	**3**	**1**	**1**		**6**	**1**	**1**
ICU-D[6]	12	4.4	**92**		**8**							
ICU-G[7]	17	6.3	**47**	**23**	**6**					**12**	**12**	
ICU-P[8]	110	11.8	**61**	**12**	**13**	**3**			**1**	**4**		**1**

Empty cells = not reported. [1]Including intensive care units (ICUs). [2]Clinics/wards, except all ICUs. [3]All ICUs. [4]T-N-E = throat-nose-ear clinics/wards (except -ICUs). [5]All ICUs (except ICU-D, ICU-G, and ICU-P). [6]ICU-D = Dermatology ICUs. [7]ICU-G = Gynaecology ICUs. [8]ICU-P = Paediatric ICUs. [9]Other species (they can differ or be absent per ward/group) are: *Candida* spp.: *C. africana* (N=32), *C. catenulata* (2), *C. intermedia* (2), *C. maritima* (3), *C. membranifaciens* (1), *C. rugosa* (3), *C. zeylanoides* (3); Non-*Candida* spp.: *Asterotremella humicola* (4),

seen from Table 1 that the different CSP and ICUs exhibit individual species-specific MIC profiles. That no significant species trends were seen may be supported by the figures based on autopsy patients with haematological malignancies, investigated from 1989 until 2008 by Lewis *et al*. (2013).

Susceptibility profiles

A prerequisite for the comparison of minimum inhibitory concentrations (MICs) throughout the complete testing period (10 years) is a reliable, reproducible and validated susceptibility testing method. This cornerstone was fulfilled because throughout all MCS the DIN 58940 mi-

the species distributions for the two investigation periods P1 (1998-2001) and P2 (2002-2008) and the complete study period (1998 to 2008) are additionally displayed.

Saccharomyces cerevisiae (n=66)	*Meyerozyma guilliermondii (n=46)*	*Magnusiomyces capitatus (n=24)*	*Yarrowia lipolytica (n=17)*	*Pichia norvegensis (n=15)*	*Debaromyces hansenii (n=10)*	*Cryptococcus neoformans (n=56)*	*Malassezia furfur (n=66)*	*Exophiala dermatitidis (n=60)*	*Trichosporon asahii (n=14)*	*Other species (n=228)*[9]	*Isolates, ST*[10] *sites/devices (n=2,348)*	*Isolates, NST*[11] *sites/devices (n=2,512)*	*MAT-ART: All liquid material*	*MAT-ART: All solid material/devices*	*MAT-ART: All swabs*
1.4	1.0	0.5	0.4	0.3	0.2	1.2	1.4	1.2	0.3	4.7	48.3	51.7	54.1	15.3	30.6
1.6	1.0	0.6	0.3	0.1	0.1	0.3	3.4	3.1	0.3	3.5	45.0	55.0	55.1	18.3	26.4
1.2	0.9	0.4	0.4	0.4	0.3	1.7	0.0	0.0	0.3	1.5	50.5	49.5	54.1	15.3	30.6
1.7	1.1	0.5	0.3	0.1	0.1	0.2	3.6	3.2	0.3	5.6	43.2	56.8	53.8	18.7	27.5
1.3	0.9	0.5	0.5	0.5	0.4	2.2	0.0	0.0	0.4	1.4	43.3	56.7	50.0	17.4	32.6
0.0	0.0	1.2	0.0	0.0	0.0	1.2				1.2	85.4	14.6	85.3	11.0	3.7
0.7	0.7	0.4	0.1	0.3	0.1	0.1				0.7	73.7	26.3	67.5	8.7	23.8
											1.1	0.3	0.7	1.3	0.3
											0.2	0.8	0.2	0.0	1.3
	4		1	1	1	1	17	16		4	1.0	13.2	1.2	24.5	9.0
						41					0.8	0.8	0.3	3.5	0.3
										4	0.1	0.8	0.0	0.4	1.3
1	1	0.4	0.1	0.5	0.3				0.2	2	26.6	18.2	27.0	12.2	19.1
4	1		0.4		0.2			0.2		5	2.6	18.3	2.5	17.9	22.0
		1	3		1	1			1	1	0.5	2.6	0.8	1.0	3.4
6	0.4	0.4				2				2	2.4	7.1	6.2	2.7	3.5
	2			2					2		1.6	0.4	0.5	4.0	0.3
0.4	1	0.2	1	0.4	0.2	0.4		0.2	0.5	5	14.8	8.9	9.4	11.2	16.7
9										5	0.5	0.9	1.2	0.1	0.1
1	1	1	1	1					1	1	12.2	6.2	10.6	6.9	7.5
	1	2		1	1				1	2	9.6	1.7	8.8	2.9	1.0
1						3				1	0.2	5.5	3.5	2.7	2.0
1	2	1							1	1	1.1	6.5	6.9	0.1	0.3
1	1	0.5	0.2	0.3	0.2	0.3				1	19.8	6.8	17.8	7.2	7.5
											0.3	0.2	0.2	0.1	0.3
											0.1	0.6	0.1	0.5	0.6
2	2	1									4.5	0.2	2.1	0.8	3.5

Cyberlindnera jadinii (2), *Kodamaea ohmeri* (1), *Malassezia globosa* (2), *M. obtusa* (1), *M. pachydermatis* (1), *M. slooffiae* (1), *M. sympodialis* (9), *Metschnikowia pulcherrima* (3), *Pichia cactophila* (2), *Pichia fermentans* (2), *Prototheca wickerhamii* (4), *P. zopfii* (23), *Rhodotorula mucilaginosa* (5), *Trichomonascus ciferrii* (2), *Trichosporon mucoides* (2),*Wickerhamomyces anomalus* (4), *Zygosaccharomyces bailii* (1).
[10]ST: Isolates from normally sterile body sites/devices. [11]NST: isolates from non-sterile body sites/devices.

crodilution method (DIN, 2002) was performed by all participating test centres. This method, established in 1996 (Schmalreck and Fegeler, 1996) ahead of the similar EUCAST method, is currently the only validated method issued for routine susceptibility testing of yeasts by an official Standardisation Organization (DIN). Except for some differences (culture media, inoculum, endpoint reading), due to its analogy to EUCAST it reveals similar results (DIN, 2007; Schmalreck *et al.*, 2012a, Schmalreck *et al.*, 2012b; Schmalreck *et al.*, 2014a) when compared to the CLSI (2008) and the EUCAST (2012) methods. The EUCAST provides the current DIN breakpoints (EUCAST, 2013) and is nowadays more frequently performed in Germany than the DIN method.

Azoles are often used for the treatment of the yeast-caused entities tested. Fluconazole and itraconazole together with the newer triazole voriconazole belong to the highly effective systemic antifungal drugs with a favourable risk-benefit ratio, and with distinct *in vitro* activity against dermatophytes, yeasts and some moulds (ITR, VOR). For this reason they are a preferred therapy option for fungal infections of skin, nails and mucous membranes. In the treatment of oropharyngeal candidiasis, candidiasis of the skin and vulvovaginal candidiasis, itraconazole and fluconazole are besides terbinafine the preferred treatment options in cases in which topical therapy has proved unsuccessful (Korting and Schöllmann, 2008). It was therefore recommended to perform the initial treatment with azoles or ciclopirox (for fungal skin infection) or, if that fails, allylamines are recommended (Hart *et al.*, 1999). In addition, the azoles, except ketoconazole, are applied for common infections in paediatrics (Allen, 2010). Thus, and since resistance to antimycotic drugs evolves, susceptibility species identification and antifungal susceptibility testing have become essential in the DGP (Czaika *et al.,* 2013) and INV settings. Emergence of resistant *Candida* and NAC isolates from paediatric wards has been reported worldwide (Shahum *et al.*, 2007; Kuzucu *et al.*, 2008; Allan, 2010; El-din El-beleidi *et al.*, 2012; Raut *et al.*, 2012; Mulu *et al.*, 2013; Verweij and Warris, 2013). In addition, triazole cross resistance of *Candida* spp. (Müller *et al.*, 2000; Chrissanthou *et al.*, 2004; Forrest, 2006; Magill *et al.*, 2006; MacGowan *et al.*, 2008; Diekema *et al.*, 2009; Van Minnebruggen *et al.*, 2010: Lewis *et al.*, 2012; Rathod *et al.*, 2012; Hill *et al.*, 3013; Verweij and Warris, 20013) and clinical implications of antimicrobial resistance for therapy (Forrest, 2006; Pfaller and Diekema, 2007; MacGowan *et al.*, 2008; Girmenia *et al.*, 2009; Van Minnebruggen *et al.*, 2010; Rathod *et al.*, 2012; Lewis *et al.*, 2012) have been well documented, together with the rapid development and appearance of fluconazole-resistant fungi by second generation triazoles during long-term treatment (Diekema *et al.*, 2009). Although *Rhodotorula* spp. is reported to play an essential role in superficial skin and mucosa infections (Zincevicience *et al.*, 2011) only a few isolates could be included from the MCS evaluations (n=5; %S* to FLC/ITR/KET/VOR: 20/71/76/100), and therefore were no longer considered in this study.

MIC assessment

Almost all susceptibility testing methods categorize the MIC results into susceptible (S), intermediate (I) and resistant (R). Today also species specific breakpoints are in use, which may result in three, or allow only one or two MIC categories (e.g., S, S and R or R alone). Therefore, depending on the breakpoints given, the intermediate categories, respectively "I"-"S", only "I", or "R"-"I" are missing or can exist instead of the complete three S-I-R results. In those cases where the MICs of different antifungal agents are concomitantly assessed, this may lead to hardly comparable or even biased comparisons. Therefore, to achieve balanced matches for microbiological comparisons and epidemiological evaluations, when breakpoints are given for S, I, and R, the intermediate category was split in such a way that 40% of the "I"-category was added to the susceptible (S*), and 60% to the resistant (R*) category. This was successfully proposed and performed for bacteria by Grimm (1991) for the introduction of a susceptibility index, and for more reliable MIC comparisons. As the intermediate category may already contain strains with mutations or regulative mechanisms on the way to resistance, the higher allotment of the intermediate category is transferred to the resistant, and the lower proportion to the susceptible category.

Additionally, this may improve evaluations and reduce assessment bias when the I-category is introduced only as "buffer zone" for antimicrobial susceptibility testings. In addition, the use of only two categories could ease the evaluation of multi-resistances because the number of possible drug combinations would be dramatically reduced (Schmalreck *et al.*, 2014b), e.g. in this paper from 3^4=81 to 2^4=16 azole combinations.

Susceptibility/resistance

According to the current species nomenclature (Species Fungorum, 2014), the listed breakpoints, and according to the complementary S*-R* categorization, the species-specific susceptibility rates (%S*) are displayed in Table 2. For further comparison purposes the median MIC ($MIC_{median} \triangleq MIC_{50}$) and the 75th percentile of the MIC (MIC_{75}) are displayed. These characteristic MIC-values are given for the complete 10-year investigation period (1998-2008), and the two artificial evaluation periods P1 (1998-2001) and P2 (2002-2008). The overall susceptibility rate (%S*) of all isolates over the 10-year testing period was for FLC, ITR, KET, and VOR: 74%, 76%, 77%, and 90%, respectively. The overall species-susceptibility was for FLC in the range of 20% (*Pichia norvegensis*) to 92% (*C. albicans*), for ITR from 17% (*Pichia norvegensis*) to 94% (*C. dubliniensis*), for KET from 29% (*Trichosporon asahii*) to 94% (*C. dubliniensis*), and for VOR from 61% (C. tropicalis) to 100% (*C. inconspicua*, *Magnusiomyces capitatus, and T. asahii*).

The ranges of susceptibility (%S*) rates in isolates from the different clinical specialities (Table 3) were for FLC: 22% (urology) to 87% (ICU dermatology), for ITR: 66% (radiology) to 95% (anaesthetics), for KET: 66% (transplantation centres) to 97% (anaesthetics), and for VOR: 62% (eye clinics) to 97% (anaesthetics). The susceptibility (%S*) ranges for isolates from the different specimen types were for FLC: 60% (solid materials/devices from paediatric wards) to 81% (stool, swabs from dermatology wards), for ITR: 69% (urine) to 83% (devices), for KET: 61% (aspirates; biopsy material) to 86% (liquids), and for VOR: 68% (solid materials/devices from paediatric wards) to 98% (devices). The overall susceptibility (%S*) to isolates from specimens of normally sterile body sites/devices was 69%, from non-sterile compartments/devices: 81%, from all liquid materials: 74%, from all solid materials: 70%, and from all swabs: 74%. Reduced susceptibility of fluconazole to all *Candida* isolates was 23% compared to 17% as reported by Leroy *et al*. (Leroy *et al*., 2009). High FLC-resistance rates (33.2%) have also been observed for the *C. parapsilosis* complex in Chinese specimens (Wang *et al*., 2012). High amounts of ketoconazole-resistant isolates (23%) were reported from countries where KET is still allowed in antifungal therapy, e.g., in an Indian hospital (Rathod *et al*., 2012) and from Malaysia (21%) (Santhanam *et al.*, 2013). Very low susceptibilities to itraconazole (56%) have been observed in Iceland (Asmundsdottir *et al.*, 2012).

In this study, the six to seven yeast species with the highest resistance rates (%R*) were for FLC: *C. sake* (86%), *P. norvegensis* (80%), *K. marxianus* (58%), *I. orientalis* (49%), *C. tropicalis* and *Ma. furfur* (46%, each), *Me. guilliermondii* (44%), *Y. lipolytica* (41%); for ITR: *P. norvegensis* (83%), *C. sake* (64%), *Ma. furfur* and *Me. guilliermondii* (61%, each), *Y. lipolytica* (59%), *S. cerevisiae* (49%), *C. glabrata*, *D. hansenii,* and *K. marxianus* (42%, each); for KET: *P. norvegensis* (53%), *Y. lipolytica* (41%), *C. tropicalis* (40%), *C. glabrata* (39%), and *M. furfur* (25%); for VOR: *C. tropicalis* (40%), *D. hansenii* and *M. furfur* (15%, each), *C. glabrata* (10%), *C. albicans* (8%), *P. norvegensis* (7%), *Me. guilliermondii* and *Y. lipolytica* (6%, each). Tables 1 and 3 demonstrate that nowadays the non-*Candida* species are under the predominant infectious agents. As reported, the incidence of infections caused by unusual organisms is likely to increase with the growing numbers of immunocompromised individuals throughout the world (Hoban *et al.,* 1999; Cisterna *et al.,* 2012). In addition, recent reports on cutaneous protothecosis appeared (Goa *et al*. 1995; Akova *et al.,* 1994; Aste, 2000; Wingard *et al.,* 2010; Troke *et al.,* 2011; Arendrup, 2013; Fothergill *et al.,* 2013). In these papers the azole susceptibilities vary, and resistance to azoles has been reported. The 30 *Prototheca* isolates belonging to an outbreak in a children´s unit in 1998 had been transmitted from pet animals (Schmalreck *et al.,* 1988) to the patients. They were completely susceptible to voriconazole and ketoconazole (100%, each),

TABLE 2 - *Characteristic MIC-values (median MIC=MICmed, the 75th percentile (MIC75), and percentage (in round figures) of susceptibility (%-S*) for the most frequently isolated yeast species (n ≥ 10), and for the isolates from commonly sterile (ST) and non-sterile (NST) body sites for the antifungal*

Investigation period	*AFA*	*Parameter*	*Candida albicans (n=2,173)*	*Candida glabrata (n=889)*	*Candida tropicalis (n=437)*	*Candida parapsilosis complex (n=293)*	*Candida dubliniensis (n=46)*	*Candida sake (n=22)*	*Candida inconspicua (n=19)*	*Other Candida spp. (n=46)[1]*	*Issatchenkia orientalis (n=328)*	*Clavispora lusitaniae (n=82)*
		$MIC_{med.}$	0.5	4.2	4.0	8.0	1.0	32.0	8.0	0.5	4.0	1.0
	FLC	MIC_{75}	1.0	16.0	32.0	32.0	2.0	32.0	16.0	2.0	16.0	2.0
Complete:		**%S***	**92.0**	**64.0**	**52.0**	**43.0**	**90.0**	**14.0**	**44.0**	**80.0**	**70.0**	**83.0**
		MIC_{med}	0.031	0.3	0.25	0.125	0.031	0.5	0.25	0.031	0.125	0.063
1998	ITR	MIC_{75}	0.063	1.0	0.5	0.25	0.063	0.5	0.5	0.031	0.25	0.125
to		**%S***	**93.0**	**52.0**	**70.0**	**72.0**	**94.0**	**58.0**	**50.0**	**83.0**	**52.0**	**86.0**
		$MIC_{med.}$	0.031	0.2	0.25	0.063	0.031	1.0	0.031	0.031	0.125	0.031
2008	KET	MIC_{75}	0.063	1.0	0.25	0.25	0.031	1.0	0.25	0.5	0.25	0.125
		%S*	**89.0**	**57.0**	**60.0**	**71.0**	**94.0**	**36.0**	**82.0**	**70.0**	**76.0**	**87.0**
N=4,860		$MIC_{med.}$	0.031	0.2	0.125	0.125	0.016	0.5	0.25	0.016	0.125	0.063
	VOR	MIC_{75}	0.031	0.5	0.5	0.25	0.031	0.5	0.125	0.031	0.25	0.063
		%S*	**93.0**	**90.0**	**61.0**	**71.0**	**98.0**	**95.0**	**100.0**	**98.0**	**99.0**	**98.0**
	n		813	339	162	131	12	9	11	39	102	40
		$MIC_{med.}$	0.5	4.0	4.0	8.0	1.0	32.0	2.0	0.25	4.0	1.0
	FLC	MIC_{75}	1.0	8.0	32.0	32.0	2.0	32.0	8.0	0.5	16.0	4.0
Period 1:		%S*	**92.0**	**73.0**	**52.0**	**40.0**	**100.0**	**11.0**	**69.0**	**91.0**	**54.0**	**83.0**
		$MIC_{med.}$	0.031	0.25	0.125	0.125	0.063	0.25	0.25	0.016	0.063	0.063
1988	ITR	MIC_{75}	0.063	1.0	0.25	0.25	0.063	0.5	0.5	0.031	0.125	1.0
to		**%S***	**93.0**	**56.0**	**68.0**	**73.0**	**100.0**	**58.0**	**53.0**	**92.0**	**82.0**	**86.0**
		$MIC_{med.}$	0.031	0.25	0.125	0.125	0.031	0.5	0.031	0.031	0.031	0.031
2001	KET	MIC_{75}	0.125	1.0	0.25	0.5	0.031	1.0	0.125	0.5	0.25	0.125
		%S*	**89.0**	**52.0**	**59.0**	**64.0**	**100.0**	**62.0**	**91.0**	**71.0**	**72.0**	**87.0**
N=1,937		$MIC_{med.}$	0.031	0.125	0.125	0.125	0.016	0.25	0.125	0.016	0.125	0.063
	VOR	MIC_{75}	0.031	0.25	0.5	0.25	0.031	0.5	0.25	0.031	0.125	0.125
		%S*	**93.0**	**95.0**	**60.0**	**70.0**	**100.0**	**100.0**	**100.0**	**97.0**	**100.0**	**98.0**
	n		1360	550	275	162	34	13	8	7	226	42
		$MIC_{med.}$	0.5	4-0	4.0	8.0	0.5	32.0	16.0	32.0	4.0	1.0
	FLC	MIC_{75}	1.0	16.0	32.0	32.0	2.0	32.0	32.0	32.0	16.0	2.0
Period 2:		**%S***	**92.0**	**64.0**	**52.0**	**45.0**	**88.0**	**14.0**	**25.0**	**16.0**	**51.0**	**86.0**
		$MIC_{med.}$	0.031	0.25	0.125	0.125	0.031	0.125	0.5	1.0	0.125	0.063
2002	ITR	MIC_{75}	0.063	1.0	0.5	0.25	0.063	0.25	1.0	8.0	0.25	0.125
to		**%S***	**93.0**	**52.0**	**68.0**	**71.0**	**95.0**	**58.0**	**45.0**	**31.0**	**75.0**	**86.0**
		$MIC_{med.}$	0.031	0.25	0.125	0.063	0.031	0.25	0.008	0.031	0.125	0.031
2008	KET	MIC_{75}	0.063	1.0	0.25	0.25	0.031	1.0	0.25	4.0	0.25	0.125
		%S*	**90.0**	**57.0**	**59.0**	**73.0**	**93.0**	**36.0**	**77.0**	**58.0**	**73.0**	**89.0**
N=2,923		$MIC_{med.}$	0.031	0.25	0.125	0.125	0.16	0.25	0.25	0.5	0.125	0.031
	VOR	MIC_{75}	0.063	0.5	0.5	0.25	0.063	0.5	0.5	1.0	0.25	0.063
		%S*	**96.0**	**93.0**	**60.0**	**71.0**	**97.0**	**95,0**	**100.0**	**100.0**	**99.0**	**99.0**

[1]Other *Candida* species: *C. africana* (N=32), *C. catenulata* (2), *C. intermedia* (2), *C. maritima* (3), *C. membranifaciens* (1), *C. rugosa* (3), *C. zeylanoides* (3); [2]Other non-*Candida* species: *Asterotremella humicola* (4), *Cyberlindnera jadinii* (2), *Kodamaea ohmeri* (1), *Malassezia globosa* (2), *M. obtusa* (1), *M. pachydermatis* (1), *M. slooffiae* (1), *M. sympodialis* (9), *Metschnikowia pulcherrima* (3), *Pichia cactophila* (2), *Pichia*

agents (AFA) fluconazole (FLC), itraconazole (ITR), ketocazole (KET) and voriconazole (VOR). The results are displayed for the study intervals 1998-2001 (Period I), 2002-2008 (Period II), and the complete study period from 1998 to 2008 (Complete).

Debaromyces hansenii (n=11)	*Kluyveromyces marxianus (n=70)*	*Meyerozyma guilliermondii (n=46)*	*Magnusiomyces capitatus (n=24)*	*Pichia norvegensis (n=15)*	*Saccharomyces cerevisiae (n=66)*	*Yarrowia lipolytica (n=17)*	*Cryptococcus neoformans (n=56)*	*Exophiala dermatitidis (n=60)*	*Malassezia furfur (n=66)*	*Trichosporon asahii (n=14)*	*Other species (n=171)*[2]	*Isolates, ST body sites/ devices (n=2,348)*	*Isolates, NST body sites/devices (n=2,512)*
4.0	8.0	8.0	1.0	16.0	4.9	2.0	1.0	1.0	8.0	2.0	2.0	1.0	1.0
4.0	16.0	8.0	2.0	16.0	8.0	64.0	4.0	4.0	32.0	8.0	4.0	8.0	8.0
60.0	**49.0**	**46.0**	**86.0**	**20.0**	**67.0**	**59.0**	**81.0**	**78.0**	**46.0**	**76.0**	**75.0**	**73.0**	**74.0**
0.125	0.25	0.25	0.125	1.0	0.25	05	0.125	0.125	0.5	0.25	0.125	0.063	0.063
1.0	0.5	1.0	0.25	2.0	1.0	8.0	0.5	0.125	1.0	0.5	0.5	0.25	0.25
58.0	**57.0**	**41.0**	**78.0**	**17.0**	**49.0**	**35.0**	**68.0**	**88.0**	**39.0**	**44.0**	**69.0**	**76.0**	**76.0**
0.063	0.031	0.25	0.2	0.5	0.031	0.25	0.031	0.125	0.063	0.25	0.031	0.125	0.031
0.5	0.125	0.5	0.25	0.5	0.125	1.0	0.125	0.125	0.25	0.5	0.25	0.25	0.125
76.0	**85.0**	**64.0**	**66.0**	**47.0**	**84.0**	**53.0**	**93.0**	**86.0**	**75.0**	**29.0**	**82.0**	**71.0**	**83.0**
0.063	0.125	0.25	0.125	0.25	0.25	0.25	0.125	0.125	0.125	0.125	0.063	0.063	0.063
1.0	0.25	0.5	0.25	0.5	0.25	1.0	0.25	0.25	0.5	0.125	0.25	0.125	0.25
87.0	**97.0**	**93.0**	**100.0**	**93.0**	**96.0**	**94.0**	**97.0**	**95.0**	**88.0**	**100.0**	**98.0**	**90.0**	**89.0**
2	19	20	11	2	31	5	5	60	66	5	95	871	1,066
2,0	8.0	8.0	1.0	16.0	1.0	64.0	0.063	1.0	8.0	1.0	2.0	1.9	1.0
4.0	16.0	16.0	2.0	16.0	8.0	64.0	0.125	4.0	32.0	1.0	8.0	4.0	4.0
70.0	**45.0**	**34.0**	**86.0**	**20.0**	**76.0**	**40.0**	**98.0**	**78.0**	**46.0**	**99.0**	**70.0**	**76.0**	**75.0**
0.063	0.125	0.5	0.125	2.0	0.25	2.0	1.0	0.125	0.5	0.25	0.125	0.063	0.063
0.125	0.5	1.0	0.25	4.0	0.5	4.0	1.0	0.125	1.0	0.25	0.5	0.25	0.25
100.0	**70.0**	**40.0**	**73.0**	**0.0**	**60.0**	**28.0**	**28.0**	**88.0**	**39.0**	**20.0**	**66.0**	**77.0**	**76.0**
0.063	0.125	0.25	0.125	0.5	0.031	0.25	0.031	0.125	0.063	0.25	0.063	0.125	0.031
0.063	0.25	0.5	0.25	0.5	0.063	1.0	0.125	0.125	0.25	0.5	0.25	0.5	0.125
100.0	**78.0**	**72.0**	**78.0**	**40.0**	**90.0**	**48.0**	**96.0**	**86.0**	**75.0**	**52.0**	**73.0**	**67.0**	**82.0**
0.031	0.125	0.25	0.125	0.25	0.063	1.0	0.125	0.125	0.125	0.125	0.063	0.063	0.063
0.063	0.25	0.5	0.25	0.5	0.125	1.0	0.25	0.25	0.5	0.25	0.25	0.125	0.125
100.0	**95.0**	**87.0**	**100.0**	**100.0**	**98.0**	**88.0**	**100.0**	**95.0**	**88.0**	**100.0**	**99.0**	**89.0**	**90.0**
9	51	26	13	13	35	12	51	0	0	9	76	1,446	1446
4.0	8.0	8.0	1,0	16.0	4.0	2.0	1.0	-	-	1.0	2.0	1.0	1.0
4.0	16.0	32.0	2.0	16.0	8.0	16.0	4.0	-	-	1.0	4.0	8.0	8.0
60.0	**50.0**	**51.0**	**86.0**	**20.0**	**63.0**	**64.0**	**79.0**	**-**	**-**	**74.0**	**82.0**	**71.0**	**74.0**
0.25	0.25	0.25	0.125	1.0	1.0	0.4	0.25	-	-	0.25	0.063	0.063	0.063
1.0	0.5	0.5	0.25	2.0	2.0	4.0	1.0	-	-	0.25	0.25	0.25	0.25
58.0	**53.0**	**43.0**	**78.0**	**17.0**	**41.0**	**36.0**	**80.0**	**-**	**-**	**40.0**	**73.0**	**74.0**	**76.0**
0.125	0.031	0.063	0.25	0.5	0.25	0.25	0.125	-	-	0.25	0.031	0.063	0.063
0.5	0.125	0.5	0.25	1.0	0.5	1.0	0.125	-	-	0.5	0.125	0.25	0.25
76.0	**86.0**	**83.0**	**66.0**	**47.0**	**78.0**	**57.0**	**92.0**	**-**	**-**	**31.0**	**86.0**	**69.0**	**83.0**
0.25	0.125	0.125	0.125	0.5	0.25	0.125	0.125	-	-	0.125	0.063	0.063	0.063
1.0	0.25	0.25	0.25	1.0	0.5	0.5	0.25	-	-	0.125	0.125	0.125	0.125
86.0	**98.0**	**97.0**	**100.0**	**93.0**	**96.0**	**100.0**	**97.0**	**-**	**-**	**100.0**	**97.0**	**89.0**	**90.0**

fermentans (2), *Prototheca wickerhamii* (4), *P. zopfii* (23), *Rhodotorula mucilaginosa* (5), *Trichomonascus ciferrii* (2), *Trichosporon mucoides* (2), *Wickerhamomyces anomalus* (4), *Zygosaccharomyces bailii* (1)

and only partly susceptible to fluconazole (*P. wickerhamii* 80%; *P. zopfii* 92%) and itraconazole (*P. wickerhamii* 40%; *P. zopfii* 76%).

When comparing the susceptibility rates (%S*) of isolates from other clinical specialities (Table 3), it turned out that the most susceptible strains were derived for FLC from: dermatological ICUs (87%), anaesthetics (85%), orthopaedics (83%), external sources (82%), gynaecology (80%), and INV associated clinics (78%); for ITR: anaesthetics (95%), (87%), ear-nose-throat clinics (83%), gynaecology (82%), external sources (81%), paediatric ICUs and INV associated clinics (80%, each); for KET: anaesthetics (97%), external source (94%), ear-nose-throat clinics (87%), INV associated clinics (85%),

TABLE 3 - *Percentage of susceptibility (%-S*, in round figures) of isolates from the different clinic specialties and compiled wards and associated ICUs when the antifungal agents (AFA) fluconazole (FLC), itraconazole (ITR), ketoconazole (KET), and voriconazole (VOR) were compared for the study periods 1998-2001*

Test period	*Time span (year) / frequency (N)*	*AFA*	*Anaesthetics (n=32)*	*Dental (n=23)*	*External (n=39)*	*Ear-Nose-Throat (n=144*	*Eye (n=21)*	*General medicine (n=1082)*	*Neurology (n=78)*	*Oncology (n=236)*	*Orthopaedics (n=49)*	*Radiology (n=34)*	*Surgery (n=443)*
Complete	**1998**	FLC	85	72	82	75	64	74	74	72	83	61	74
	to	ITR	95	79	81	83	75	78	78	72	76	66	74
	2008	KET	97	83	94	87	69	75	81	79	78	75	73
	N=4,860	VOR	97	87	92	88	62	91	90	93	92	85	88
Period 1	**1998** to **2001** N=1,937	n	0	16	12	79	15	478	10	66	28	23	140
		FLC	-	78	92	72	60	76	72	77	84	63	80
		ITR	-	76	100	83	69	79	74	72	83	77	81
		KET	-	81	100	87	56	72	80	85	76	83	75
		VOR	-	88	100	87	53	90	90	93	89	87	89
Period 2	**2002** **To** **2008** N=2,923	n	32	7	27	65	6	604	68	170	21	11	303
		FLC	97	60	76	80	73	73	74	70	82	60	70
		ITR	95	86	72	85	83	77	79	71	68	44	71
		KET	97	86	92	87	100	78	81	76	82	56	73
		VOR	97	86	89	88	83	91	90	94	95	82	87
Simple statistics for all 4 antifungal agents (n=4) and total isolates (N=4,860)													
Parameters for MIC-values:													
Investigation Period 1 (P1)													
Investigation Period 2 (P2)													
Statistical methods for MIC-values and SIR-assessments													
Parameter for P1 – P2													
Spearman correlation													
Gamma													
Stuart's Tau-c													
Kappa, simple													
Friedmann's Chi-Square Test (stratified) with output Cochran-Mantel-Haenszel (CMH, Based on Rank Score)													
CMH Nonzero Correlation													
CMH Row Mean Scores Difference													

No isolates from this period.

dental clinics (83%), urology (84%), and gynaecology (82%); and for VOR: anaesthetics (97%), gynaecology (94%), oncology (93%), external sources and orthopaedics (92%, each), general medicine and transplantation (91%, each), SYS- and DGP-associated clinics (90%, each). Thereof, the isolates with the highest susceptibility (%S*) rates (lowest resistance rates) were associated with the following specimens for FLC: devices (84%), dermatological materials (82%), stool and dermatological swabs (81%, each), gynaecological swabs (80%); for ITR: devices (83%), dermatological and gynaecological swabs (81%, each), stool (60%), catheters and blood (79%, each); for KET: liquids (86%), catheter, stool, and gynaecological swabs (84%,

(Period 1), 2002-2008 (Period 2), and the complete study time from 1998 to 2008 (Complete). In addition, the minimal statistics for the two test periods and a statistical comparison of the MIC-values and their SIR-assessments for the two periods are given.

Transplantation (n=269)	*Urology (n=188)*	*Dermatology (N=356)*	*Gynaecology (N=519)*	*Paediatrics (N=473)*	*SYS (n=1,063)*	*INV (n=493)*	*DGP (n=1,448)*	*ICIUs (n=635)*	*ICU, Dermatology (n=12)*	*ICU, Gynaecology (n=17)*	*ICU, Paediatrics (n=110)*
74	22	63	80	70	74	76	71	75	87	77	76
75	79	67	82	74	74	79	75	78	70	71	80
66	84	80	82	74	74	85	79	76	70	81	81
91	88	87	94	88	90	87	90	88	75	88	84
148	117	236	213	274	405	249	723	57	4	2	19
79	80	65	83	76	79	76	72	79	75	100	77
75	79	67	81	74	77	79	77	85	50	50	84
60	84	81	82	72	72	84	77	74	50	100	89
94	86	88	95	90	91	86	91	81	50	50	95
121	71	120	306	299	658	244	725	578	8	15	91
67	77	61	77	69	71	76	71	74	88	73	74
75	76	68	83	74	72	79	75	77	80	71	78
74	84	81	83	76	72	85	78	77	80	79	79
86	90	86	93	87	90	89	90	89	93	93	82

Characteristic MIC-values

MIC_{range}	MIC_{mean}	MIC_{mode}	ECV_{median}	MIC_{50}	MIC_{75}	MIC_{90}	Resistant
mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	%
0.008-128.0	0.017	0.031	0.5	0.125	0.5	4.0	14.0
0.008-128.0	0.016	0.031	0.5	0.125	0.5	4.0	15.0

		MIC			**SIR**
DF	**Value**	**Pro./ASE**	**DF**	**Value**	**Pro./ASE**
	0.0815	0.0115		-0.0091	0.0112
	0.0707	0.0092		-0.0195	0.0241
	0.0618	0.0084		-0.0057	0.0070
	-	-		0.0037	0.0086
1	51.4095	<.0001	1	0.6437	0.4224
15	591.8255	<.0001	2	2.0495	0.3589

each), for VOR: devices (98%), gynaecological swabs (94%), liquids, solid materials, and catheter (91%), gynaecological materials (90%).

When comparing the susceptibility (%S*) of isolates, derived from normally sterile body sites (ST) to others from non-sterile (NST) sites, the overall susceptibility was equal for FLC and ITR (73%/78%), whereas that of KET and VOR was slightly higher (71% vs. 83% / 84% vs. 90%). The fact that the susceptibility rates shown here may sometimes appear lower than in other recent publications can be due to several reasons:

1) The culture medium used according to the DIN method (DIN, 2002) may lead to slightly, up to 1 log2-dilution higher MIC values (DIN, 2007);
2) The endpoint readings were made as visual detection of no growth or to be at least a 80% growth reduction, compared to the 50% growth inhibition as detected by photometric means (EUCAST, 2013). Arendrup (2013) demonstrated that using a less stringent end-point of 50% growth inhibition rather than of 80% inhibition will result in a one-step difference in MICs;
3) Adhering to the new nomenclature for yeast species (Schmalreck *et al.*, 2014a), the former large *Candida* species group is split into many new species with mostly higher MIC profiles. In addition, the azoles demonstrate *in vivo* a clinical efficacy of around 75%, with varying ranges, depending on antifungal agent, patient collective and treated pathogens (from a minimum of 50%-60% to a maximum efficacy of 67% to 85%) (Walter *et al.*, 1983; Smith and Henry, 1984; Korting *et al.*, 1992; Akova *et al.*, 1994; Goa *et al.*, 1995; Aste, 2000; Sobel *et al*, 2001; Troke *et al.*, 2011; Akhtar *et al.*, 2012). When high numbers of isolates, derived from patients with severe underlying diseases are tested these ranges may also be reflected *in vitro*. Therefore, the 75th percentile (Table 2) of the MIC (where at least 75% of the isolates should be inhibited) comes about to the mean value of the reported *in vivo* efficacy. In addition it turned out that MIC_{75} may be close to the epidemiological cut-off value (Tables 2 and 3). A better approach to evaluate azole efficacy, to predict clinical failure could be therefore considering the MIC_{75}, instead of using MIC_{50} and MIC_{90} comparisons. This may apply at the moment for all available antifungal agents, of which none is reported in clinical studies to demonstrate an *in vivo* activity of ≥80%.

Microbiology trends

For potential trend analysis the complete 10-year test period (CP) was split into two approximate periods, Period I (P1: 1998 to 2001), and Period II (P2: 2002 to 2008). The mean susceptibility (%) of the most frequently isolated species from 1998 to 2008 was 67% (FLC), 64% (ITR), 83% (KET), and 87% (VOR). The mean susceptibility of the isolates of P1 was 66% (FLC), 55% (ITR), 84% (KET), and 88% (VOR), and for P2 64% (FLC), 58% (ITR), 78% (KET), and 87% (VOR). Considering MICs, the characteristic MIC-values, susceptibility rates, and the amount of multi-resistance (Tables 2 and 3) were of similar magnitude and statistically not significantly different (Table 3). Although some minor differences (species and azole specific) may be seen in the tables when P1 and P2 are compared, despite the increased use and consumption of the azoles, and the concomitant report of new drug resistance mechanisms (Berry *et al.*, 2011; Cowen *et al.*, 2012; Quito-Alemany *et al.*, 2012; Singh-Babak, *et al.*, 2012; Hill *et al.*, 2013; Mayer *et al.*, 2013; Spampinato and Leonardi, 2013), especially for fluconazole. No proof was found for a significant trend regarding an increase/decrease of azole resistance between P1 and P2 over the complete study time (Tables 2 and 3; Figures 1-3). The characteristic MICs of the isolates from different clinical specialities followed the reports from several published studies, which found no difference in distribution of *Candida* species in ICUs and conventional wards, and if, very little variation was determined in fluconazole susceptibility among isolates of *C. albicans*, *C. tropicalis* and *C. parapsilosis,* respectively, no increase in fluconazole resistance at all (Wang *et al.*, 2012; Asmundsdottir *et al.*, 2012; Kohli *et al.*, 2002; Chen *et al.*, 2003; Marchetti *et al.*, 2004; Sandven *et al.*, 2006; Charlier *et al.*, 2006; Havlickova *et al.*, 2008; Chen *et al.*, 2012; Yamaguchi *et al.*, 2012). Among others, Friedman's test was applied, where there is a significant difference of the MIC/SIR-levels between the different clin-

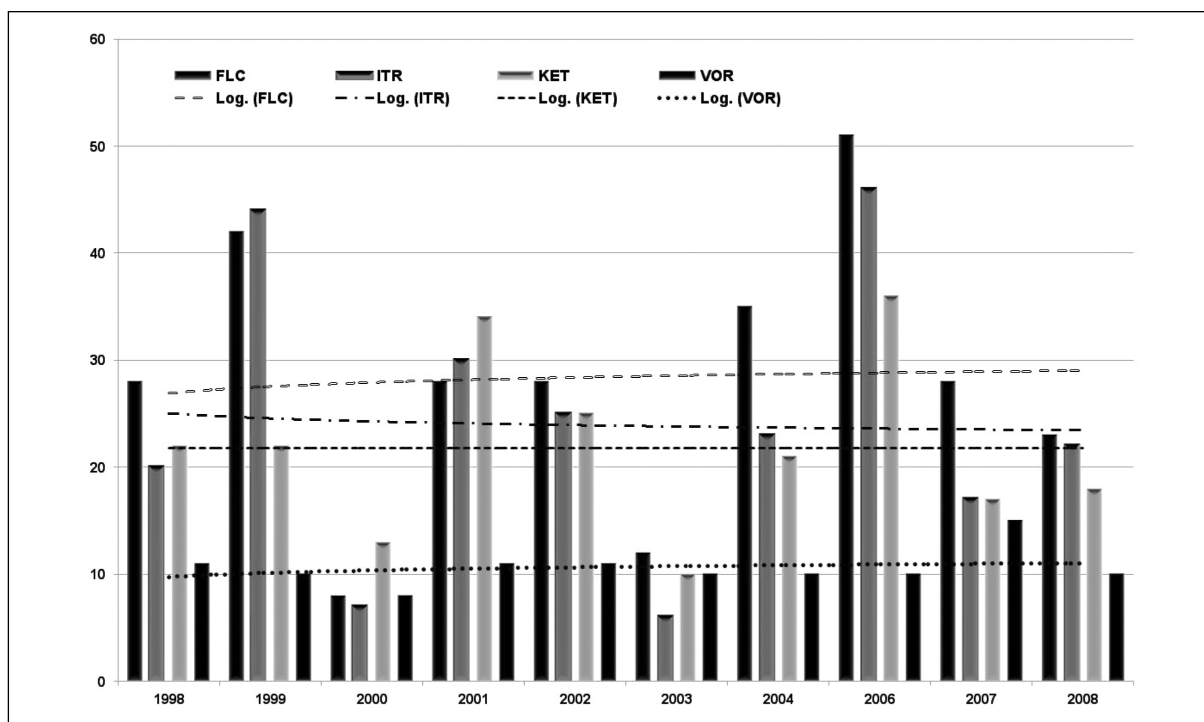

FIGURE 1 - *Resistance rates (%R*) of all isolates (n=4,860) to the azoles fluconazole (FLC), itraconazole (ITR), ketoconazole (KET), and voriconazole (VOR) over the 10-year investigation period (1998 to 2008; Log. = logarithmic trend lines).*

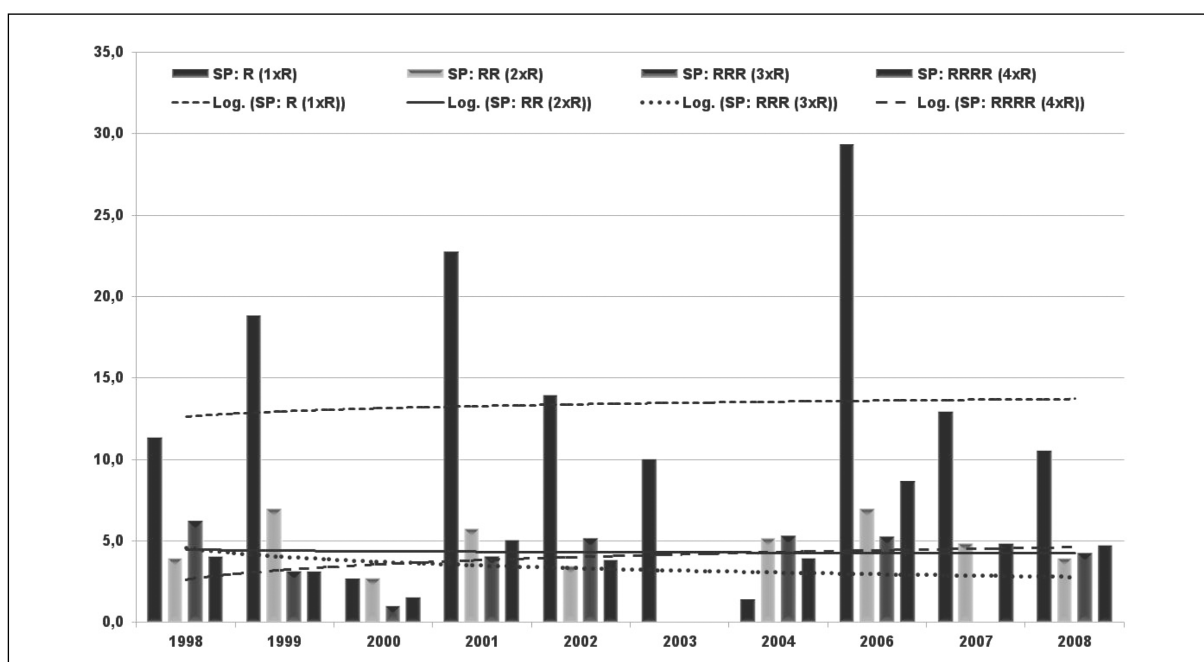

FIGURE 2 - *Percentage (%) of populations of all isolates with single (1xR), or parallel-resistances (nxR) to the azoles: fluconazole (FLC), itraconazole (ITR), ketoconazole (KET), and voriconazole (VOR) over the 10 year investigation period (1998 to 2008; Log. = logarithmic trend lines).*

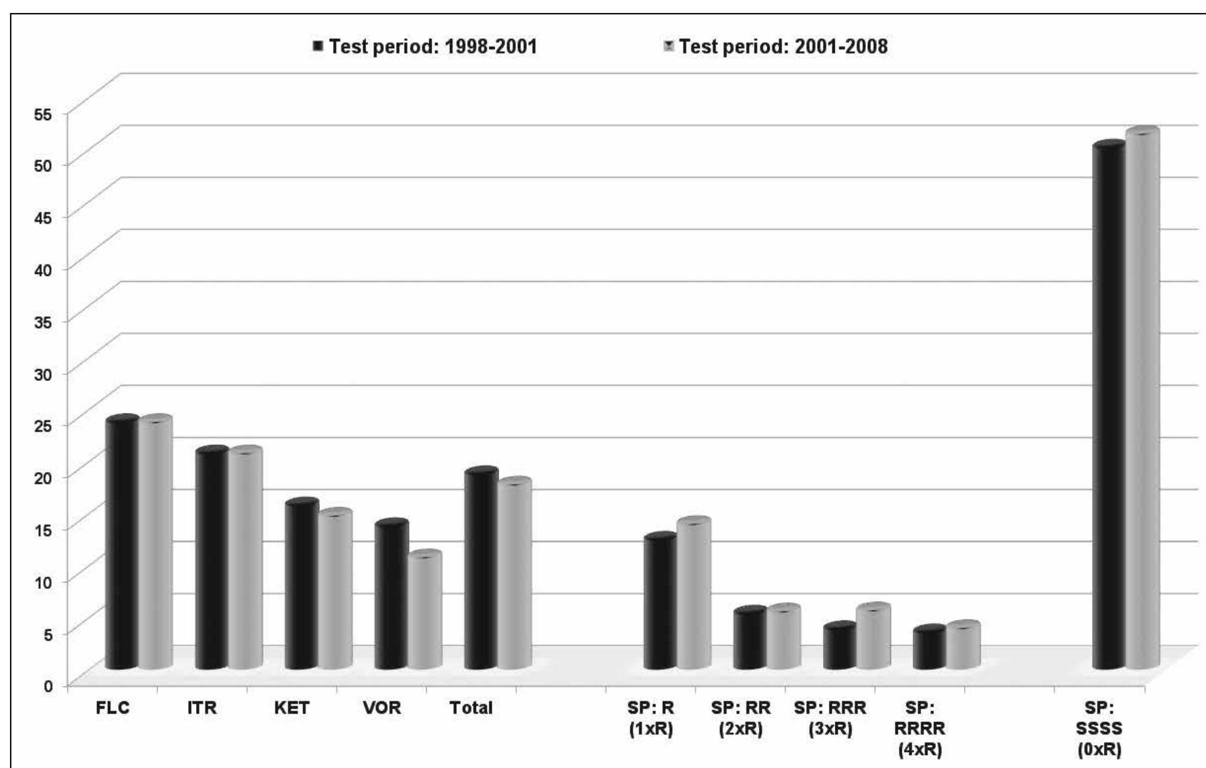

FIGURE 3 - *Resistance rates (%R*) of fluconazole (FLC), itraconazole (ITR), ketoconazole (KET), and voriconazole (VOR) as determined by S-I-R classification of MICs, and the percentage (%) of populations from all isolates estimated by SPA with single-resistances, multiple resistances (parallel-resistance) to all azoles for the test periods 1998-2001 and 2002-2008.*

ical specialities and evaluation periods (Table 3), showing that the overall differences in MIC, SIR assessments for the two test periods were statistically not significant.

In addition, characteristic MIC values, derived of azole *in vitro* studies around the world (only investigations have been considered where both, the MIC_{50} and MIC_{90} were reported (Chrissanthou, 1998; Sugizaki *et al.*, 1998; Pfaller *et al.*, 2001; Cuenca-Estrella *et al.*, 2002; Durán *et al.*, 2003; Cuenca-Estrella *et al.*, 2004; Hajjeh *et al.*, 2004; Takakura *et al.*, 2004; Therese *et al.*, 2006; Kuzucu *et al.*, 2008; Arendrup *et al.*, 2011; Pemán *et al.*, 2012; Czaika *et al.*, 2013; Czaika *et al.*, 2013; Mulu *et al.*, 2013), and compared to the data determined (Table 6). Except for some small deviations, it can be clearly seen from Tables 2 and 3 that the characteristic MIC values obtained within the reported investigation periods are very similar, and they do not change significantly over the complete study periods covering 1991 to 2010. This is particularly interesting because the worldwide studies were performed partly with quite different susceptibility testing methods. However, the combined effects of fast growth rates to a high number of cells at the infection site, and genetic processes of mutation and selection account for the extraordinary rates of adaptation and resistance development, not only in bacteria but also in fungi. The fact that the ability to exchange genes is much lower in fungi (yeasts) (Hof, 2008) may contribute to the still high susceptibility rates for antifungal drugs over reported the long time investigation periods.

Parallel and cross resistance

In general, cross resistance in the literature comprises the resistance of different anti-infective drugs to an isolate, regardless of their belonging to the same or different chemical substance classes. As this may be misleading in MIC assessments, it is proposed to use for this evaluation "parallel resistance", i.e. resistance

to two or more agents of the same substance class, and "cross resistance", as resistance to two or more drugs of different substance classes. Parallel resistance among azoles can be expected as their interactions within fungi share the same targets. The development of antifungal drug resistance evolving by different mechanisms during antifungal treatment has potential clinical impact (Cisterna *et al.*, 2012; Pfaller, 2012; Hill *et al.*, 2013), and a few other factors may significantly contribute to multi-drug resistance such as adaptive mutagenesis (Quito-Alemany *et al.*, 2012), environmental stress (Berry *et al.,* 2011; Cowen and Steinbach, 2012; Singh-Babak *et al.*, 2012), the host immune response to *Candida* Lewis *et al.,* 2012), and *Candida* pathogenicity mechanisms (Mayer *et al.,* 2013). Several mechanisms of azole resistance have been described in *Candida* species (Panackal *et al.,* 2006; Cannon *et al.,* 2009; Chackrabarti, 2011; Silva *et al.,* 2011; Vandenputte *et al.,* 2012; Farahyar *et al.,* 2013; Spampinato and Leonardi, 2013; Verweij and Warris, 2013), which all may contribute to parallel-, respectively cross-resistance, such as:

- Lack of drug penetration due to changes in membrane lipids and sterols;
- Failure to accumulate drugs intracellular by activation of efflux proteins (pumps), which may be caused particularly from overexpression of the *CDR1*, *CDR2*, *PDH1*, and *MDR1* genes;
- Upregulation of the transcription factors *UPC2* and *NTD80*;
- Upregulation and overexpression of several reductases and oxidoreductases;
- Overexpression of genes coding for the azole target enzyme (lanosterol 14α-demethylase);
- Point mutations in genes coding for the target enzyme, reducing azole target cell affinity (e.g. mutation of ERG11, decreased ERG11p affinity).

Overexpression of efflux proteins and associated increased efflux-pump activities are considered to be the most relevant mechanism of azole resistance, conferring parallel resistance to several azoles (Cannon *et al.*, 2009). Molecular mechanisms of resistance (Pfaller *et al.,* 2010; Spampinato and Leonardi, 2013), namely the different resistance mechanisms indicate that more than one of them may be present in a given strain or strain population, and they may be executed single, in sequence, stepwise or together, and they may have additive effects, leading to cross resistance (Pfaller and Diekema, 2007; Pfaller, 2012). It is also suggested that multi-drug resistance in yeast is closely linked to the status of membrane lipids, wherein the overall drug susceptibility phenotype of an isolate appears to be an interaction between diffusion of the AFA into the cell, activation and activity of the efflux pumps, and the membrane lipid layers (Mukhopadhyay and Kohli, 2002). Additionally, clinically significant azole (cross) resistance is mainly due to either acquired resistance in commensal opportunistic fungal pathogens or to the selection of strains within species that show intrinsic resistance. Considering all the above-mentioned factors it is obvious that the evolution of multi-drug antifungal resistance is constantly progressing and, if present, should be detected by appropriate analysis methods. In addition, it has been shown that phylogenetic relationships matter (Schmalreck *et al.,* 2014a). The evolution of drug resistance is therefore a ubiquitous phenomenon with a profound impact on human health, which narrows the limited range of antifungal drugs (Hill *et al.,* 2013). Therefore a proposed way to overcome this shortage is reported to be combination therapy (Hill *et al.,* 2013; Spampinato and Leonardi, 2013). For this strategy the effectiveness of the individual drugs and their drug resistance profile is a prerequisite. However, direct proof of cross resistance cannot be obtained only by the S-I-R categorization of the MICs. Due to the additive character of assessing the monovalent susceptibility results, the corresponding susceptibility patterns are hidden. Therefore multi-resistance of azoles (strain or population-specific), namely, the determination of parallel resistance of azoles in this paper was determined by an adapted resistance pattern analysis for bacteria (Fegeler *et al.*, 1998; Schmalreck *et al.*, 2014b), adapted for fungi. Hereby, the antifungal activities of the tested drugs (assessed MICs) are broken down individually for each species by a pair to pair analysis of the assigned susceptibility classes for each azole and each strain. Thus for the four antimycotics and the three assess-

ment categories, 3^4=81 individual susceptibility pattern (SP) can theoretically occur, actually however, only 60 different SPs have been detected in the 4,860 yeast isolates (74% of the theoretical). Table 5 displays the pattern of (species) populations where:

1) each azole was tested as susceptible (SP: SSSS);
2) the populations which revealed only one azole as resistant (SP: R);
3) those with two or more azoles were tested as resistant (SP: RR, RRR, or RRRR=two to four times azole parallel/multi-resistant).

Thus, of all isolates (n=4,860), a population of 13.7% (n=668) harboured only a single resistance to one azole, 6.7% (n=325) a twofold, 4.4% (n=213) a threefold, and 4% (n=196) a fourfold parallel-resistance (Table 4). The clinic speciality-specific and the investigation period-specific multi-resistance profiles are laid out in Table 6.

The different species show different patterns of multi-resistance. Species with the most frequent fourfold resistances were (% species related): *C. tropicalis* (13.3%;n=58), *Debaryomyces hansenii* (9.1%; n/N=1/11), *C. parapsilosis* (8.9%; n=26), *Malassezia furfur* (6.1%; n/N=4/66), *Meyerozyma guilliermondii* (4.4%; n/N=2/46), *C. glabrata* (4.1%; n=36), *Saccharomyces cerevisiae* (3%; n/N=2/66) and *Candida albicans* (3%;n=64), *Exophiala dermatitidis* (1.7%; n/N=1/60), and *Issatchenkia orientalis* (0.6%; n =2). Thus, according to the "new" nomenclature, in four of the remaining "true" *Candida*, in three of the "new" non-*Candida*, and in three known not-*Candida* species, the fourfold azole parallel resistance could be determined.

TABLE 4 - *The number (N) and percentage (%) of populations with resistance to a single (SP: R = 1xR) antifungal agent (AFA) or with parallel resistance to two (SP: RR = 2xR) or more azoles (SP: RRR = 3xR, r SP: RRRR = 4xR) in clinical yeast species (N > 10) are displayed together with the populations, hich show susceptibility all of the four AFAs (SP: SSSS = 0xR) for the SP-basis: fluconazole (FLC),*

Yeast isolates → *Susceptibility Pattern (SP)* *SP-basis*:*				*Total isolates*	*Candida albicans*	*Candida dubliniensis*	*Candida glabrata*	*Candida inconspicua*	*Candida parapsilosis*	*Candida sake*	*Candida tropicalis*	*Other Candida species[1]*	*Clavispora lusitaniae*
FLC	*ITR*	*KET*	*VOR*	*N=4,860*	*2173*	*46*	*889*	*19*	*293*	*22*	*437*	*46*	*82*
Individual SP-profile:				*Number / percentage (%) of strains per species with single or multiple resistance*									
S	S	S	S	2488/51.2	1738/80.0	41/89.1	183/20.6	3/15.8	83/28.3	0/0.0	135/30.9	26/56.5	52/63.4
R	v	v	v	301/6.2	14/0.6	2/4.3	66/7.4	5/26.3	68/23.2	11/50.0	39/8.9	2/4.4	1/1.2
v	R	v	v	186/3.8	5/0.2	0/0.0	90//10.1	1/5.3	3/1.0	0/0.0	1/0.2	1/2.2	5/6.1
v	v	R	v	125/2.6	17/0.8	0/0.0	87/9.8	0/0.0	1/0.3	0/0.0	0/0.0	9/19.6	1/1.2
v	v	v	R	56/1.5	35/1.6	0/0.0	3/0.3	0/0.0	6/2.0	0/0.0	12/2.7	0/0.0	0/0.0
R	R	v	v	95/2.0	2/0.1	1/2.2	34/3.8	1/5.3	5/1.7	0/0.0	2/0.5	3/6.5	1/1.2
R	v	R	v	44/0.9	1/0.05	0/0.0	19/2.1	1/5.3	7/2.4	6/27.2	1/0.2	0/0.0	1/1.2
R	v	v	R	79/0.6	18/0.8	0/0.0	9/1.0	0/0.0	25/8.5	0/0.0	24/5.5	1/2.2	0/0.0
v	R	R	v	70/1.4	0/0.0	0/0.0	65/7.3	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0
v	R	v	R	5/0.1	3/0.1	0/0.0	0/0.0	0/0.0	1/0.3	0/0.0	0/0.0	0/0.0	0/0.0
v	v	R	R	32/0.7	15/0.7	0/0.0	1/0.1	0/0.0	1/0.3	0/0.0	15/3.4	0/0.0	0/0.0
R	R	R	v	81/1.7	1/0.05	1/2.2	52/5.9	0/0.0	0/0.0	2/9.1	0/0.0	2/4.4	1/1.2
v	R	R	R	11/0.2	8/0.4	0/0.0	2/0.2	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0
R	v	R	R	93/1.9	15/0.7	0/0.0	4/0.4	0/0.0	21/7.2	0/0.0	52/11.9	0/0.0	0/0.0
R	R	v	R	28/0.6	4/0.2	0/0.0	5/0.6	0/0.0	5/1.7	0/0.0	11/2.5	0/0.0	0/0.0
R	R	R	R	196/4.0	64/3.0	0/0.0	36/4.1	0/0.0	26/8.9	0/0.0	58/13.3	0/0.0	0/0.0

[1]Other *Candida* spp.: *C. africana* (N=32), *C. catenulata* (2), *C. intermedia* (2), *C. maritima* (3), *C. membranifaciens* (1), *C. rugosa* (3), *C. zeylanoides* (3); [2]Other Not-*Candida* spp.: *Asterotremella humicola* (4), *Cyberlindnera jadinii* (2), *Kodamaea ohmeri* (1), *Malassezia globosa* (2), *M. obtusa* (1), *M. pachydermatis* (1), *M. slooffiae* (1), *M. sympodialis* (9), *Metschnikowia pulcherrima* (3), *Pichia cactophila* (2), *Pichia fermentans* (2), *Prototheca wickerhamii* (4), *P. zopfii* (23), *Rhodotorula mucilaginosa* (5), *Trichomonascus ciferrii* (2), *Trichosporon mucoides*

The fact that significant cross resistance between azoles exists, especially to "older" azoles was reported by Panackal *et al.* (2006). Complete azole cross resistance in isolates from invasive yeast infections have been described (Pfaller and Diekema, 1998; Müller *et al.*, 2000; Diekema *et al.*, 2009; Pfaller, 2012; Vandenputte *et al*, 2012). Aside of 26.9% VOR-resistant *C. albicans* isolates, Orrù *et al.* (2008) found that 11.5% of the dental patients with oral thrush harboured FLC-KET-VOR cross-resistant isolates. It is also reported that due to diverse resistance mechanisms and the extensive use of fluconazole, and to some extent of itraconazole for prophylaxis regimens, resistance and azole cross resistance can develop simultaneously (Pfaller and Diekema, 2007; Pfaller, 2012). Not to be underestimated is the selection pressure due to excess fluconazole use, which may play a major role in triazole cross resistance (Hof, 2001; Schlatter, 2003; Serfling *et al.*, 2007; Müller *et al.*, 2007; Chen *et al.*, 2012; Heusinkveld *et al.*, 2013). Indeed, triazoles used as fungicides were found to induce cross resistance of azoles used in human therapy (Snelders *et al.*, 2012), namely 'azole pre-exposure affects *Aspergillus fumigatus* populations in patients' (Alanio *et al.*, 2012). The possible sequential rise of azole MICs by stepwise mutations can be another cause of parallel/cross resistance (Kohli *et al.*, 2002). Therefore, and depending on the resistance mechanisms activated, elevated MICs may be detected (Quito-Alemany *et al.*, 2012). However, reported evidence of higher cross resistance rates may also originate from changing (lowering) breakpoints for MIC assessment in order to achieve better approach from *in vitro* testings to the clinical situation (Cisterna *et al.*, 2012).

itraconazole (ITR), ketoconazole (KET) and voriconazole (VOR). SPs with intermediate "I" or susceptible "S" are indicated aside of the resistant "R" tested azoles by "v". Only "I" or "S" tested strains or susceptibility patterns (SPs) with individual combinations of S-I or I-R (empty fields under SP-sequence) are not considered, respectively displayed in this table.

Debaromyces hansenii	*Kluyveromyces marxianus*	*Magnusiomyces capitatus*	*Malassezia furfur*	*Meyerozyma guilliermondii*	*Issatchenkia orientalis*	*Pichia norvegensis*	*Saccharomyces cerevisiae*	*Yarrowia lipolytica*	*Exophiala dermatitidis*	*Cryptococcus neoformans*	*Trichosporon asahii*	*Other yeast species*[2]
11	*70*	*24*	*66*	*46*	*328*	*15*	*66*	*17*	*60*	*56*	*14*	*171*
(parallel resistance) according to the indicated SP-profile (individual SP)[3]												
4/**36.4**	16/**22.9**	8/**33.3**	15/**18.8**	6/**13.0**	90/**27.4**	1/**6.7**	14/**21.2**	3/**17.7**	31/**51.7**	26/**46.4**	1/**7.1**	73/**42.7**
0/**0.0**	11/**15.7**	**1/4.2**	10/**15.2**	2/**4.4**	62/**18.9**	2/**13.3**	0/**0.0**	0/**0.0**	2/**3.3**	2/**3.6**	1/**7.1**	1/**0.6**
2/**18.2**	4/**5.7**	0/**0.0**	14/**21.2**	7/**15.2**	8/**2.4**	3/**20.1**	14/**21.2**	1/**5.9**	0/**0.0**	7/**12.5**	3/**21.4**	22/**12.9**
0/**0.0**	1/**1.4**	1/**4.2**	0/**0.0**	0/**0.0**	6/**1.8**	0/**0.0**	0/**0.0**	1/**5.9**	0/**0.0**	0/**0.0**	0/**0.0**	1/**0.6**
0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	2/**1.2**
0/**0.0**	4/**5.7**	0/**0.0**	6/**9.1**	3/**6.5**	18/**5.5**	5/**33.3**	3/**4.6**	3/**17.7**	0/**0.0**	1/**1.8**	0/**0.0**	4/**2.3**
0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	1/**2.2**	6/**1.8**	0/**0.0**	0/**0.0**	0/**0.0**	1/**1.7**	0/**0.0**	0/**0.0**	1/**0.6**
0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	2/**3.3**	0/**0.0**	0/**0.0**	0/**0.0**
0/**0.0**	0/**0.0**	0/**0.0**	1/**1.5**	1/**2.2**	2/**0.6**	0/**0.0**	1/**1.5**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**
0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**
0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**
1/**9.1**	1/**1.4**	0/**0.0**	4/**6,1**	2/**4.4**	6/**1.8**	1/**6.7**	0/**0.0**	4/**23.5**	0/**0.0**	0/**0.0**	0/**0.0**	2/**1.2**
0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	1/**0.6**
0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	1/**0.3**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**
0/**0.0**	2/**2.9**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	0/**0.0**	1/**1.8**	0/**0.0**	0/**0.0**
1/**9.1**	0/**0.0**	0/**0.0**	4/**6.1**	2/**4.4**	2/**0.6**	0/**0.0**	2/**3.0**	0/**0.0**	1/**1.7**	0/**0.0**	0/**0.0**	0/**0.0**

(2),*Wickerhamomyces anomalus* (4), *Zygosaccharomyces bailii* (1); [3]For space reasons SPs containing only "I" and "S" tested isolates are for table-space reasons not displayed. Therefore the total percentage per species may not accomplish 100%. *SP-basis, pre-defined azole-sequence: FLC-ITR-KET-VOR.

TABLE 5 - *Number and percentage (in round figures) of species isolated from blood cultures (BC) together with their species-specific susceptibility pattern profiles. Except of those isolates susceptible to all azoles (SP: SSSS), only the SP-profiles are displayed where fluconazole (FLC), itraconazole (ITR), ketoconazole (KET), and voriconazole (VOR) were assessed as resistant (R). The SPs containing azoles assessed as "I" or "S" are indicated as variable ("v") within the SP-profile. Therefore the total number of the SP-R-profiles (n=520) is lower than the total number of species derived from the blood cultures (n=587), and the individual species totals (row-%) may not achieve 100%.*

Susceptibility pattern-"R" profiles (n=520)→*			*Blood culture Isolate Susceptibility Patterns (SP basis: FLC-ITR-KET-VOR)*															
			Number (n) and percentage (%) of actual SP-profiles (SPs containing only "S" and "I" sequences are for space reasons not displayed) (R ≙ appropriate resistant azole of SP-basis)															
			Rvvv	*vRvv*	*vvRv*	*vvvR*	*vvRR*	*vRRv*	*RvRv*	*RvvR*	*vRvR*	*RRvv*	*RvRR*	*RRRv*	*RRvR*	*vRRR*	*RRRR*	*SSSS*
Total BC isolates / SPs	*N*	*%*	*32*	*11*	*86*	*1*	*4*	*4*	*16*	*4*	*1*	*5*	*25*	*13*	*2*	*1*	*24*	*291*
	587	*100*	*5*	*2*	*35*	*0.2*	*0.7*	*0.7*	*3*	*0.7*	*0.2*	*1*	*4*	*2*	*0.3*	*0.2*	*4*	*50*
BC species	**N**	%	n/%N	n/%N	n/%N	n/%N	n/%N	n/%N	n/%N	n/%N	n/%N	n/%N	n/%N	n/%N	n/%N	n/%N	n/%N	n/%N
Candida albicans	293	49.9	2/**1**		48/**16**		3/**1**			1/**0.3**	1/**0.3**		5/**2**		2/**1**	1/**0.3**	9/**3**	216/**74**
Candida dubliniensis	2	0.3																2/**100**
Candida glabrata	87	14.8	2/**2**	7/**8**	20/**23**			3/**6**	4/**5**			1/**1**	1/**1**	7/**8**			3/**4**	12/**14**
Candida inconspicua	5	0.9	1/**20**	2/**40**														
Candida parapsilosis	56	9.5	5/**9**		7/**13**	1/**2**		1/**2**	10/**18**	2/**4**			8/**14**				6/**11**	10/**18**
Candida rugosa	2	0.3	1/**50**									1/**50**						
Candida tropicalis	60	10.2	1/**2**		11/**18**		1/**2**		1/**2**	1/**2**			11/**18**				4/**7**	27/**45**
∑ *Candida* spp.	505	86.0	12/2	9/2	86/17	1/0.2	4/1	4/1	15/3	41	1/0.2	2/0.4	25/5	7/1	2/0.4	1/0.2	22/**4**	267/53
Asterotremella humicola	2	0.3																1/**50**
Clavispora lusitaniae	14	2.4												1/**7.1**				8/**57**
Debaromyces hansenii	3	0.5															1/**33**	1/**33**
Geotrichum candidum	2	0.3																2/**100**
Issatchenkia orientalis	36	5.1	14/**39**	1/**3**					1/**3**			2/**6**		3/**8.3**			1/**3**	6/**17**
Kluyveromyces marxianus	10	1.7	2/**20**											1/**10.0**				2/**20**
*Magnusiomyces capitatus***	1	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Meyerozyma guilliermondii	11	1.9	4/**36**	1/**9**								1/**9**		1/**9.1**				2/**18**
Saccharomyces cerevisiae	1	0.2																1/**100**
Cryptococcus neoformans	2	0.3																1/**50**
∑ Non-*Candida* spp.	82	14.0	20/24	2/2					1/1			3/4		6/7			2/2	24/29

** Only SP-profiles without R, respectively, only SPs with "S" and "I" assessed azoles detected

When the azole parallel resistance (twofold to fourfold) within the different clinical speciality groups (CSG) is examined by SPA, no significant differences in the resistance patterns could be detected. However, when the species with fourfold parallel resistance are considered, they were derived in different amounts from the different wards. Most of the complete azole-resistant isolates (% of total 4xR) could be assigned to the following aggregated clinics: ICU-DGP (6.5%), INV (5.1%), ICU (4.4%), DGP (4.0), SYS (3.9%) and GM (3.1%).

Unlike the completely azole-resistant isolates (SP: RRRR), populations without any resistant or intermediate tested strains (0xR; SP: SSSS) are frequently encountered (Table 5; n=2,488/51% of total). The highest amount (% in rounded figures) of these populations was found among: *Candida albicans* (80%) followed by *Clavispora lusitaniae* (63%), *Exophiala dermatitidis* (51%), *Cryptococcus neoformans* (46%), *Debaryomyces hansenii* (36%), *Magnusiomyces capitatus* (33%), *Candida tropicalis* (31%), *Candida parapsilosis* (28%), and *Issatchenkia orientalis* (27%). In addition, the group of "other" *Candida* species (*C. africana*, *C. catenulata*, *C. intermedia*, *C. maritima*, *C. membranifaciens*, *C. rugosa*, *C. zeylanoides*) contained a population of 37% (n=46). A population of 54% (n=171) with complete susceptibility (SP: SSSS) to all azoles was detected in the group of "other" non-*Candida* species (*Asterotremella humicola*, *Cyberlindnera jadinii*, *Kodamaea ohmeri*, *Malassezia globosa*, *M. obtusa*, *M. pachydermatis*, *M. slooffiae*, *M. sympodialis*, *Metschnikowia pulcherrima*, *Pichia cactophila*, *Pichia fermentans*, *Prototheca wickerhamii*, *P. zopfii*, *Rhodotorula mucilaginosa*, *Trichomonascus ciferrii*, *Trichosporon mucoides*, *Wickerhamomyces anomalus*, *Zygosaccharomyces bailii*).

From Table 4, it also can be seen that the assessment of MICs into S, I, and R cannot be used alone to determine single or multiple resistance within an individual isolate or in strain populations. For example, among the 4,860 isolates, no population could be detected by SPA to harbour an intermediate tested azole, likely to be received by SIR assessment with the listed or other breakpoints. Therefore, by S-I-R categorization alone, the percentage of the intermediate tested isolates ranged between 8% and 15% for the different wards. Additionally, the percentage of susceptible classified isolates is far higher (74%-79%) in contrast to SPA (48%-61%). This is because no population can be detected by S-I-R assessment alone, which may harbour only isolates that are susceptible in parallel to all azoles tested (SP: SSSS). The same holds true for the populations with diverse individual single and/or multiple resistances (for space reasons not shown here), which however, can be distinguished by SPA (Table 4).

Blood cultures

By comparing the susceptibilities (%S*) of isolates from blood cultures derived from the different clinical specialities, respectively those originating from severe systemic fungal infections (Van Minnebruggen *et al*., 2010) with those from superficial fungal infections, the isolated species again show varying susceptibility pattern profiles (Table 5). The 82 (14%) non-*Candida* species are differently distributed among the different clinical specialities (DGP/n=12, SYS/12, INV/1, GM/30, ICU/17). *Only C. albicans*, *C. glabrata*, and the *C. parapsilosis* complex were recovered from all sites. The *Candida* species isolated from 1997 until 2009 (n=505, 10.4% of total strains) had low MIC-values, except *C. dubliniensis* and *C. albicans* (Table 5). However, 10% of the *C. albicans* strains were resistant to the azoles. This is in contrast to Hoban *et al*. (1999), who could not demonstrate fluconazole-resistant isolates in their blood cultures. However, when the currently available breakpoints appropriate to the test method would have been used, these and other former results should definitely be higher, as demonstrated by Fothergill *et al*. (2013). The SP-populations (except single resistances) demonstrated various different individual SP-profiles from two to fourfold parallel resistance (PR) in the range of 0.3% to 50% for the different *Candida* species, and from 3% to 39% PR for the NCS isolates. Fourfold PR was determined in *C. albicans*, *C. glabrata*, *C. parapsilosis*, C. tropicalis, *D. hansenii*, and *I. orientalis*. Interestingly, in 29% of the NAC and in 53% of the *Candida* species BC-populations with complete azole susceptibility (SP: SSSS) could be determined (Table 5).

TABLE 6 - *Comparison of yeast susceptibility profiles (mg/L: median MIC=MIC50, 90th percentile of MIC=MIC90, and tested range of MIC=MICrange)*

Investigation period	*Strains tested*	*Characteristic MIC-values (mg/L)*				
		FLC_{50}	FLC_{90}	FLC_{range}	ITR_{50}	ITR_{90}
1991-1994	222	0.06	5.5	0.03-8.0	-	-
1994-1998	123	0.25	0.25	0.125-1.0	0.125	0.25
1996-1999	514	0.5	16.0	0.063-128.0	0.063	0.5
1996-1999	230	0.5	8.0	0.125-128.0	0.031	0.5
1997-1999	589	0.25	16.0	-	-	-
1997-1999	161	0.25	4.0	-	-	-
1997-1999	132	0.25	4.0	-	-	-
1997-1999	302	0.25	8.0	-	-	-
1998-2000	935	0.5	8.0	0.125-128.0	0.063	0.5
1997-2001	53	0.5	16.0	0.125-32.0	0.063	0.25
1998-2001	**1937**	**1.0**	**32.0**	**0.063-128.0**	**0.063**	**1.0**
1999-2002	50	3.3	51.2	0.2-102.4	-	-
2001-2002	535	0.5	32.0	0.125-128	-	-
1991-2003	1348	0.5	16.0	0.25-64.0	-	-
2002-2003	351	0.39[a)]	8.0	0.125-128.0	0.03*	0.25
2003-2004	13	2.0	8.0	0.063-8.0	0.031	0.5
2004-2005	561	0.5	16.0	0.125-128.0	0.125	1.0
2005-2006	100	0.25	4.0	0.125-256.0	0.031	0.5
2002-2007	495	1.0	32.0	0.125-64.0	0.125	2.0
2004-2007	5821	0.5	8.0	0.125-256.0	-	-
2007-2008	638	0.5	16.0	0.125-128.0	0.031	1.0
2007-2008	420	0.25	2.0	0.063-64.0	≤0.016	0.063
2002-2008	**2923**	**1.0**	**32.0**	**0.063-128.0**	**0.063**	**1.0**
2008-2008	90	0.25	2.0	0.125-64.0	0.016	0.063
1997-2009	9627	1.0	32.0	0.063-128.0	0.063	1.0
2004-2009	64	4.0	32.0	0.125-32.0	0.125	1.0
2009-2010	1374	0.5	8.0	0.008-256.0	0.125	0.5

No data available/not reported; t. p. = this publication; [a)]Geometric mean of MIC; AR=Argentina; BR=Brazil; CD=Canada; DE=Germany; DK=Denmark; ET= Ethiopia; IN=India; IT=Italy; JP=Japan; LM=Latin America; NO=Norway; SP=Spain; SW=Sweden; TK=Turkey; US=U.S.A. [References 1-20]. 1: (Sugizaki *et al.*, 2012); 2: (Chrissanthou *et al.*, 1998); 3: Cuenca-Estrella *et al.*, 1993); 4: (Pfaller *et al.*, 2001);

CONCLUSIONS

The high prevalence of superficial mycotic infections shows that 20–25% of the world's population has skin mycoses, thus being one of the most frequent forms of infection. The epidemiological trend in skin mycoses worldwide is paralleled by changes in nosocomial and invasive fungal infections.

As shown for dermatology, gynaecology and paediatric wards, the yeast species and species distribution are similar to the other clinical specialities compared, dealing with systemic or invasive infections. This is connected with a significant shift in the distribution of the infection-causing agents as reported in the literature, and enhanced due to the shift towards non-*Candida* species when the currently valid taxonomy is applied. As the overall aetiology has not changed during the time period of the multicentre studies from 1997 to 2009, formerly called infections with atypical, rare or "cryptic" yeast (*Candida*) isolates now possess "new" taxonomic denominations, e.g. such as *Issatchenkia orientalis*, *Meyerozyma guilliermondii*, *Kluyveromyces marxianus*, *Clavispora lusitaniae*, *Debaryomyces hansenii*, or *Yarrowia lipolytica* (see Panel). As shown, they have

listed from the appropriate literature (Origin) for different investigation periods.

							Origin	
ITR_{range}	KET_{50}	KET_{90}	KET_{range}	VOR_{50}	VOR_{90}	VOR_{range}	Country	Reference
-	0.03	3.0	0.03-4.0	-	-	-	BR	[1]
0.008-0.5	-	-	-	-	-	-	SW	[2]
0.016-16.0	-	-	-	-	-	-	SP	[3]
0.016-16.0	-	-	-	-	-	-	AR	[3]
-	-	-	-	0.016	0.25	-	US	[4]
-	-	-	-	0.016	0.125	-	CD	[4]
-	-	-	-	0.016	0.125	-	LM	[4]
-	-	-	-	0.008	0.25	-	EU	[4]
0.016-8.0		-	-	-	-	-	US	[5]
0.016-2.0	-	-	-	-	-	-	SP	[5]
0.008-16.0	**0.063**	**1.0**	**0.016-16.0**	**0.063**	**0.5**	**0.008-16.0**	**DE**	t. p.
-	0.2	0.8	0.025-12.8	-	-	-	IN	[6]
-	-	-	-	-	-	-	JP	[7]
-	-	-	-	-	-	-	NO	[8]
0.016-8.0	-	-	-	0.02*	0.25	0.016-16.0	SP	[9]
0.031-64.0	-	-	-	-	-	-	TK	[10]
0.031-16.0	-	-	-	0.031	0.5	0.031-16.0	DE	[11]
0.008-32.0	-	-	-	0.008	0.063	0.008-4.0	BR	[12]
0.008-16.0	0.031	2.0	0.008-16.0	0.008	0.5	0.008-16.0	IT	[13]
-	-	-	-	0.031	0.25	0.008-16.0	US	[14]
0.008-16.0	-	-	-	0.016	0.25	0.008-16.0	IT	[15]
≤0.016-0.5	-	-	-	≤0.016	0.031	≤0.016-2.0	AR	[16]
0.008-16.0	**0.031**	**1.0**	**0.016-16.0**	**0.063**	**0.5**	**0.008-16.0**	**DE**	t. p.
0.016-16.0	2.0	4.0	0.016-16.0	-	-	-	ET	[17]
0.008-16.0	-	-	-	0.016	0.5	0.008-16.0	DE	[18]
0.031-8.0	-	-	-	0.125	0.5	0.031-4.0	DK	[19]
0.016-16.0	-	-	-	0.016	0.25	0.008-4.0	SP	[20]

5: (Hajjeh *et al.*, 2004); 6: (Durán *et al.*, 2014); 7: (Therese *et al.*, 2006); 8; (Takakura *et al.*, 2004); 9: (Sandven *et al.*, 2006); 10: (Cuneca-Estrella *et al.*, 2005); 11: (Kuzucu *et al.*, 2008); 12: (Motta *et al.*, 2010); 13: (Asticcioli *et al.*, 2009); 14: Lyon *et al.*, 2010); 15: (Morace *et al.*, 2011); 16: (Córdoba *et al.*, 2011); 17: Mulu *et al.*, 2013); 18: (Schmalreck *et al.*, 2014a); 19: (Arendrup *et al.*, 2011); 20: (Pemán *et al.*, 2012).

emerged in all clinical specialities and associated ICUs, and been markedly amplified by the taxonomic changes according to the "one fungus one name" principle.

The increase in resistant isolates may be due to the recently aligned and lowered breakpoints. Aside from the species profiles, the clinical specialities demonstrate their specific susceptibility/resistance profiles, at least for the tested azoles. Most of the species derived from both systemic and superficial infections treated in different clinic specialities still have a high percentage of complete azole-susceptible populations (SYS; 80%, P1: 79%, P2: 77%) / non-SYS; 81%, P1: 80%, P2: 79%) which may come to a steady state due to the limited action of the currently available antifungal agents. As detected by SPA, no statistically significant trends, in terms of an increase or decrease of azole resistance in isolates, originated from clinics/wards dealing with systemic or superficial infections was observed during a 10-year evaluation period. The disclosed and also elsewhere reported observation of emergence of uncommon/rare yeast species with specific MIC-profiles and resistance patterns underline the importance of proper identification of the isolates by applying the new taxonom-

ic changes and denominations. However, the appearance of new species solely by applying the new nomenclature should not be misinterpreted as the emergence of new aetiological agents, because as shown, they were already present at the beginning of these evaluations. In addition, it could be demonstrated that the sole assessment of MICs into the categories S, I and R is not sufficient to detect appropriately single or multi-resistances. Therefore, MIC assessments should be improved significantly for more reliable evaluations and for a better approach from the *in vitro* to the *in vivo* situation. Improvement of susceptibility testing with appropriate data mining and analyses, e.g. coupled with susceptibility pattern/cluster analysis, may be useful to assist in the appropriate choice of drug for a calculated therapy, respectively for selection of appropriate antifungal drug combinations to combat infections by yeasts and yeast-related aetiological agents.

Panel

New nomenclature (Schmalreck *et al.*, 2014) of "old" yeast species (**in brackets**) mentioned in this paper:

- ***Asterotremella humicola*** (*Candida humicola*)
- ***Clavispora lusitaniae*** (*Candida lusitaniae*)
- ***Cyberlindnera jadinii*** (*Pichia jadinii*)
- ***Debaryomyces hansenii*** (*Candida famata*)
- ***Geotrichum candidum*** (*Dipodascus capitatus*)
- ***Hanseniaspora uvarum*** (*Kloeckera apiculata*)
- ***Issatchenkia orientalis*** (*Candida krusei*)
- ***Kluyveromyces marxianus*** (*Candida kefyr*)
- ***Magnusiomyces capitatus*** (*Blastoschizomyces capitatus*, *Saprochaete capitata*)
- ***Metschnikowia pulcherrima*** (*Candida pulcherrima*)
- ***Meyerozyma guilliermondii*** (*Candida guilliermondii*)
- ***Pichia fermentans*** (*Candida lambica*)
- ***Pichia norvegensis*** (*Candida norvegensis*)
- ***Trichomonascus ciferrii*** (*Candida ciferrii*)
- ***Wickerhamomyces anomalus*** (*Candida pelliculosa*, *Hansenula anomala,* and *Pichia anomala*)
- ***Yarrowia lipolytica*** (*Candida lipolytica*)
- ***Zygosaccharomyces bailii*** (*Saccharomyces elegans*)

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