Candida and *Fusarium* sepsis in Serbian pediatric and neonatology settings The role of blood culture (BC)

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INVASIVE FUNGAL INFECTIONS - IFI

Aspergillus spp.

Candida spp.

(causes ~80% IFI)

Cryptococcus spp.,

Pneumocystis spp.,

Fusarium spp.,

(causes ~ 20% IFI)

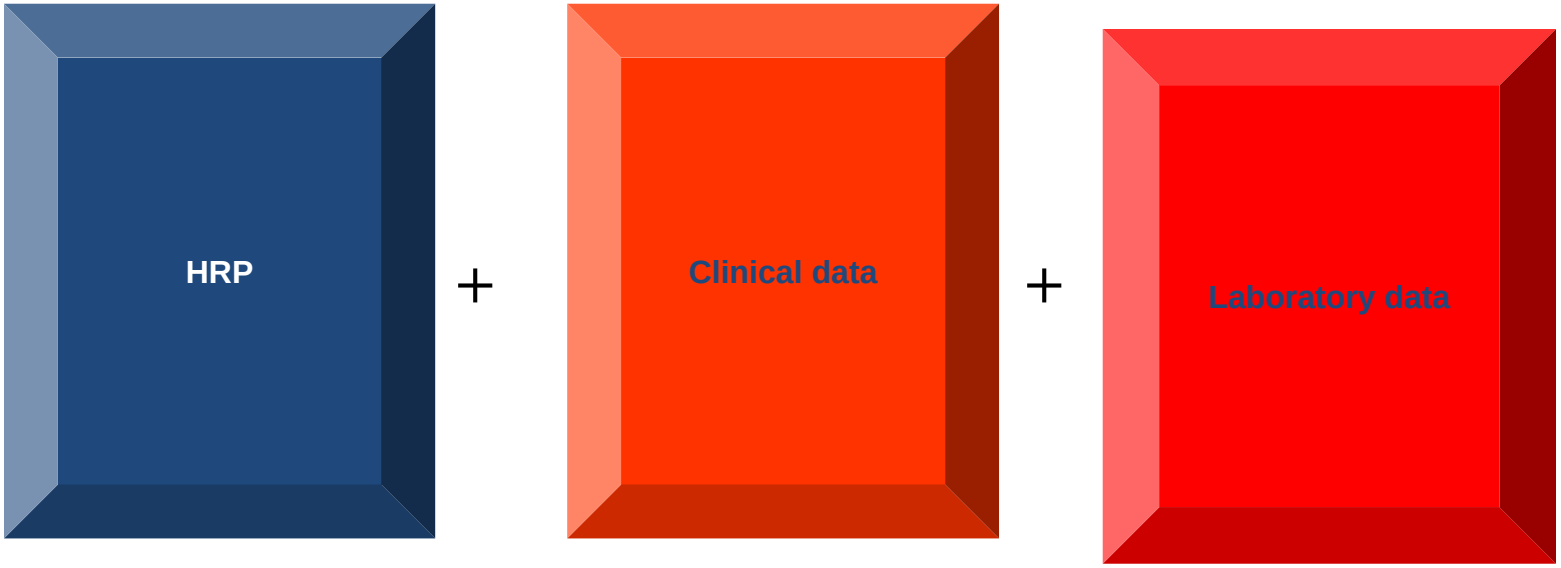
Mucorales rare fungi

(causes ~ 1% IFI)

IMPORTANT– CLINICAL PRESENTATION OF IFI USUALLY IS NOT SPECIFIC

Early diagnosis of IFI is crucial

IFI requires personalized approach



High risk patients (HRP) for IFI

Neutropenia

Central venous catheter

Parenteral nutrition

Wide spectrum antibiotics

Neonates – low body mass

CMV infection

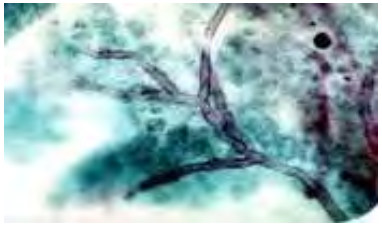
…

A fungal bloodstream infection (BSI)

- systemic inflammatory response syndrome (SIRS)
- severe sepsis
- septic shock

Misdiagnosing, mistreating or late initiation of appropriate therapy commonly results in a high mortality rate in patients with fungla BSI in Serbia.

Microscopy (cytology)



RAPID, CHEAP, PROVEN FI

Low sensitivity

Invasive sampling procedure

Expert is needed for detection

Expert interpretation is needed

Culture

CHEAP, PROVEN FI,

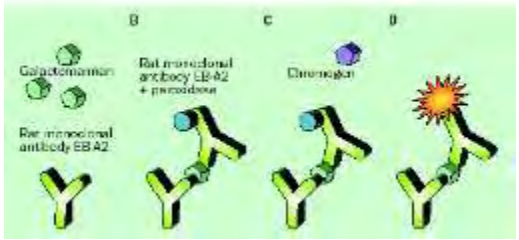
SUSSCEPTIBILITY TESTING

FUNGAL IDENTIFICATION

Slow method, low sensitivity,

Invasive sampling procedure

Ag/Ab



RAPID, PROBABLE FI

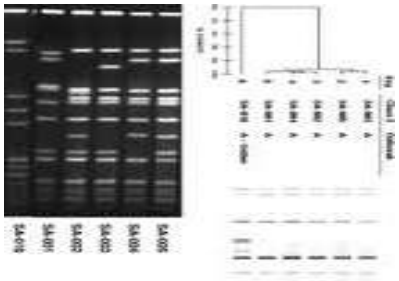
HIGH SENSITIVITY

HIGH PPV (Ab)

HIGH NPV (Ag)

Interpretation

DNA/RNA



RAPID, PROBABLE FI

HIGH SENSITIVITY

Contamination

Interpretation

Tissue +++ (invasive sampling)

Laboratory diagnosis of IFI: Cytology-culture

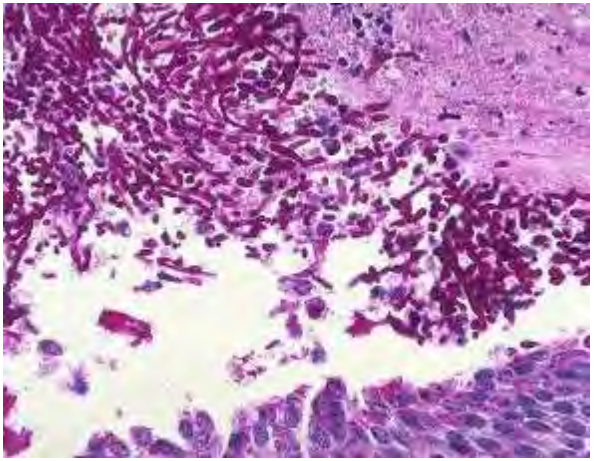
Cytology **Mycology culture**
(different media and temperatures)

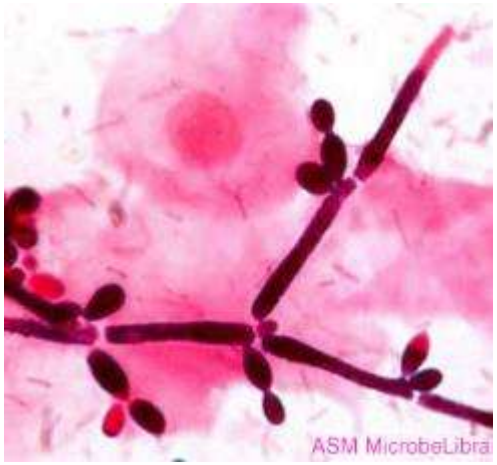
Cytology: detection of fungal elements
(difficult identification of fungal genus)

Mycology culture - simple

Adventage: other moulds could be isolated: *Fusarium* spp., Mucorales or *Scedosporium* spp.

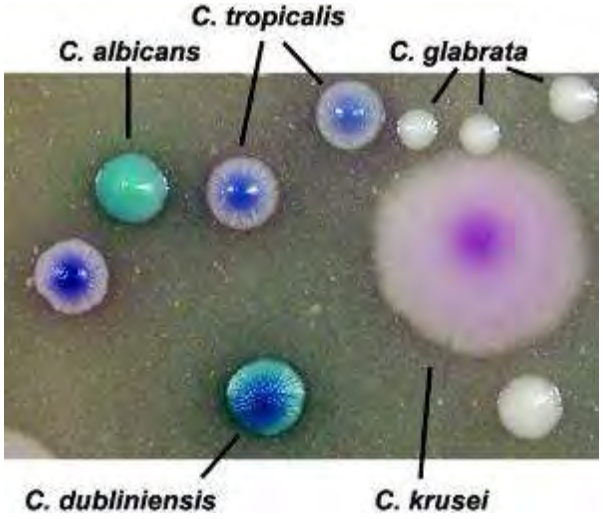
PAS and *C. albicans* in kidney





Gram staining and *Candida* spp. in vaginal swab

Candida spp. on Chrom agar



Blood culture (BC)

In a fungal BSI viable fungi are present in blood and BC are sensitive to detect these low amounts of fungi.

Fungal density varies during the course of disease;

BC diagnostics yield positive results in about 50% of cases;

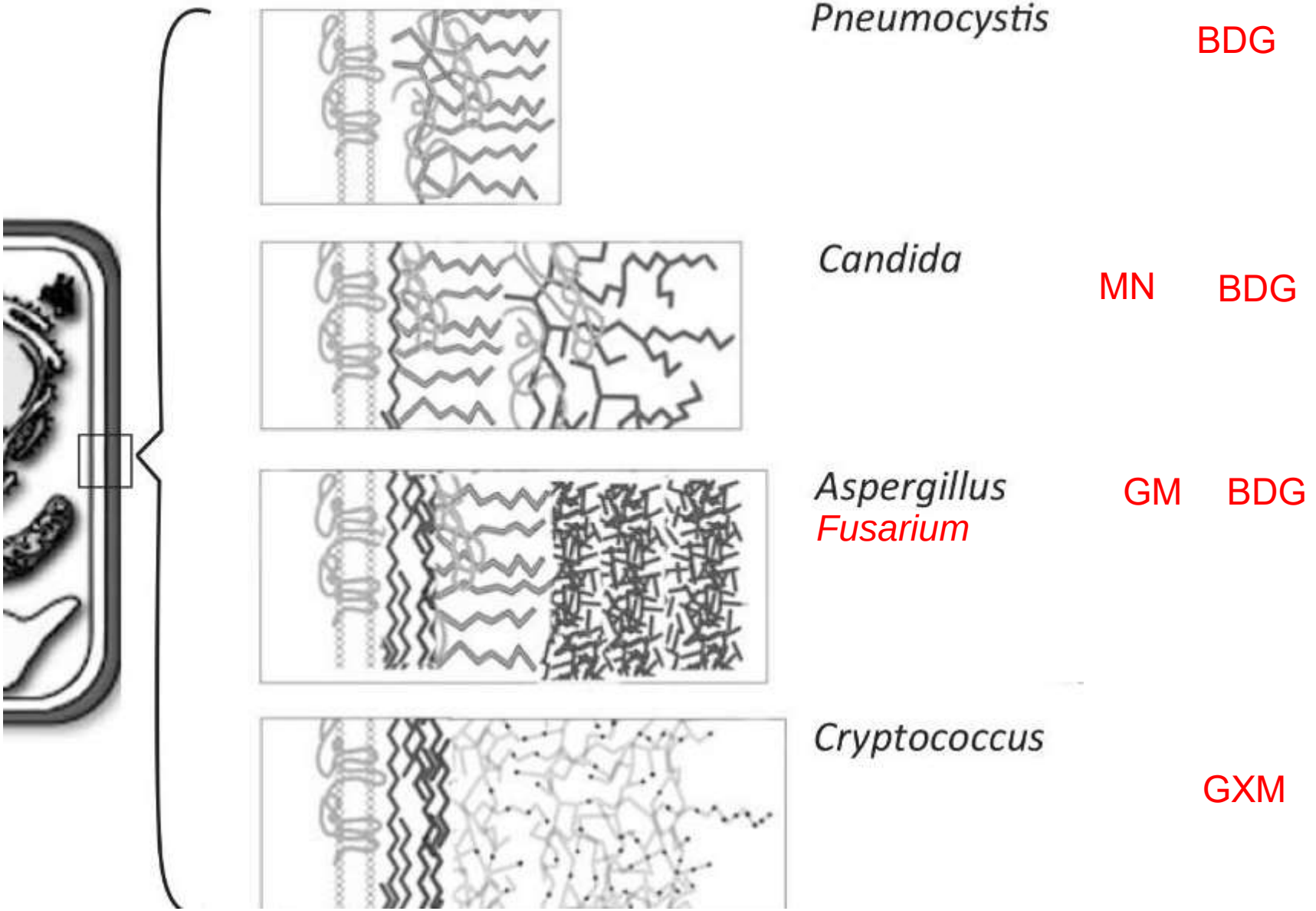
Positive BC means the proven fungal BSI

Traditionally, in Serbian pediatrics and neonatology settings the BC is focusing on bacteria

Fungal BSI become more and more important – BC makes it detection possible

Biomarkers and molecular techniques have been developed to replece BC

Biomarkers and molecular techniques **have not** replaced BC and it is still not clear how to best integrate them in diagnostic pathways.


Pneumocystis
BDG
Candida
MN
BDG
Aspergillus
Fusarium
GM
BDG
Cryptococcus
GXM

Fusarium sepsis and nosocomial outbreak in Serbian pediatric setting

*Fusarium**

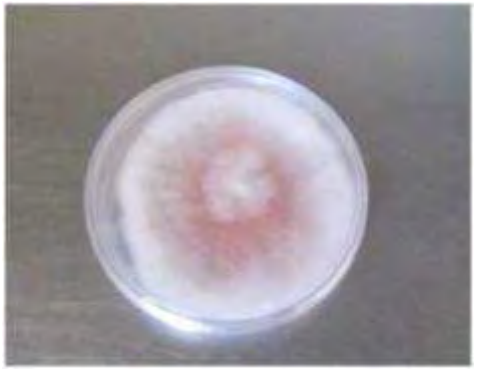
Emerging pathogen for Serbia

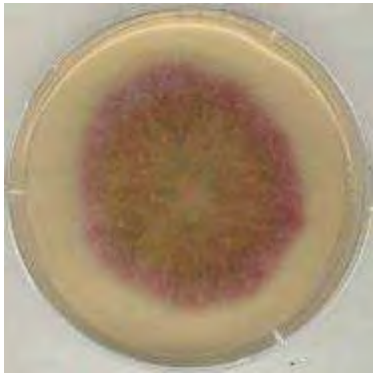
Frequent "water borne" epidemic

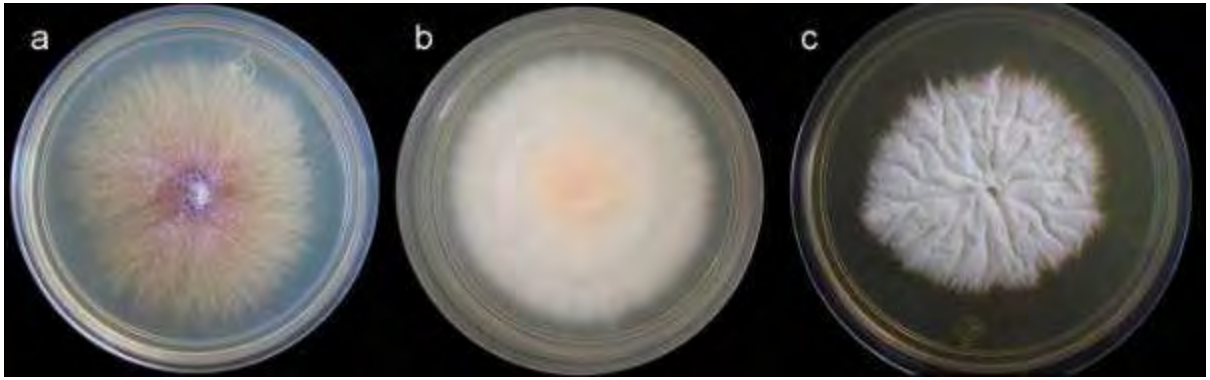
Human infections and species:

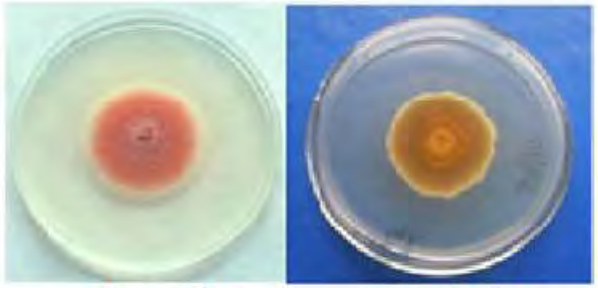
- *F. solani* (~50%)
- *F. oxysporum* (~20%)
- *F. verticillioides* (~10%)
- *F. moniliforme* (~10%)

Fusarium – different species and media

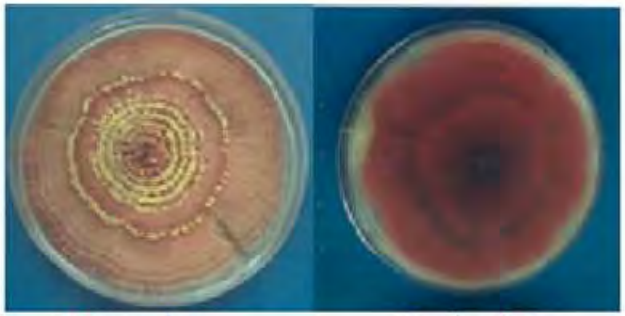






Front plate Back plate

Fusarium lateritium



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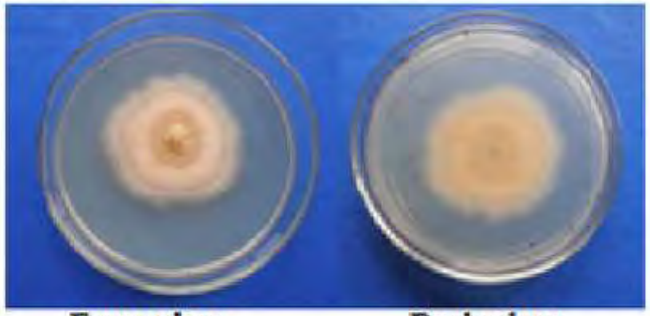
Fusarium decemcellulare



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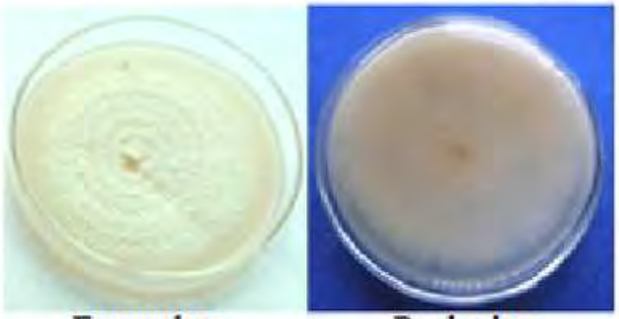
Fusarium xylarioides



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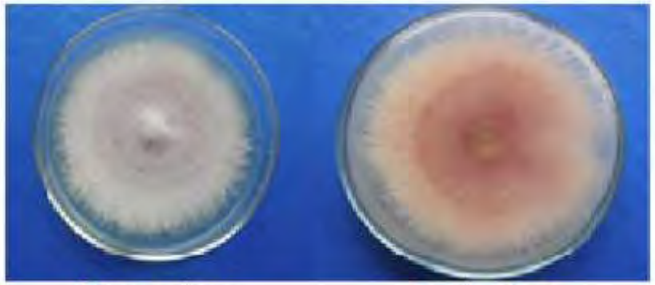
Fusarium solani



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Fusarium oxysporum

Front plate Back plate

Fusarium pallidoroseum

Fusarium sp.

Any mold isolations from BC – RL MM

GM POSITIVE IN 9/11 *FUSARIUM* CASES

TABLE 1 Serum GM index results for patients with disseminated/deep-seated *Fusarium* infection[a]

Patient	Sex	Age (yr)	Predisposing factor(s)	*Fusarium* infecting isolate	Positive biological sample(s)	Serum GM index range
1	M	29	Allo-HSCT	*F. oxysporum*	Pleural fluid	0.69–0.90
2	M	63	Leukemia	*F. oxysporum*	Bronchial secretions	1.40–2.30
3	M	55	AML	*F. proliferatum*	Blood	0.89–0.86
4	F	61	Allo-HSCT	*F. proliferatum*	Blood	1.37–2.33
5	F	56	AML, allo-HSCT	*F. proliferatum*	Blood	0.7–2.15
6	M	8	Non-Hodgkin's lymphoma	*F. proliferatum*	Blood	0.53–7.7
7	M	19	ALL, auto-HSCT	*F. proliferatum*	Blood	0.54–1.45
8	F	57	Postchemotherapy aplasia, RAEB	FSSC	Purulent nasal discharge	0.50–0.60
9	M	41	Non-Hodgkin's lymphoma, allo-HSCT	*F. verticillioides*	Skin biopsy	Negative
10	M	5	ALL	*F. verticillioides*	Blood	Negative
11	F	9	ALL, auto-HSCT	*F. verticillioides*	Blood	0.70–4.16

[a] M, male; F, female; allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; RAEB, refractory anemia with excess blasts; auto-HSCT, autologous hematopoietic stem cell transplantation; FSSC, *Fusarium solani* species complex.

Tortorano AM et al Cross-reactivity of *Fusarium* spp. in the Aspergillus Galactomannan enzyme-linked immunosorbent assay. J Clin Microbiol. 2012;50:1051-3.

Tortorano AM, Prigitano A, Esposto MC, Arsic Arsenijevic V, Kolarovic J, Ivanovic D, Paripovic L, Klingspor L, Nordøy I, Hamal P, Arikan Akdagli S, Ossi C, Grancini A, Cavanna C, Lo Cascio G, Scarparo C, Candoni A, Caira M, Drogari Apiranthitou M; ECMM Working Group. European Confederation of Medical Mycology (ECMM) epidemiological survey on invasive infections due to Fusarium species in Europe. Eur J Clin Microbiol Infect Dis. 2014;33:1623-30.

Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-014-2111-1

ARTICLE

European Confederation of Medical Mycology (ECMM) epidemiological survey on invasive infections due to *Fusarium* species in Europe

A. M. Tortorano · A. Prigitano · M. C. Esposto · V. Arsic Arsenijevic · J. Kolarovic · D. Ivanovic · L. Paripovic · L. Klingspor · I. Nordoy · P. Hamal · S. Arikan Akdagli · C. Ossi · A. Grancini · C. Cavanna · G. Lo Cascio · C. Scarparo · A. Candoni · M. Caira · M. Drogari Apiranthitou · On the behalf of the ECMM Working Group

Table 1 Contribution of different countries to the European Confederation of Medical Mycology (ECMM) *Fusarium* Working Group

Country	Collected cases			
	Total	Disseminated infection		Localised infection
		Proven	Probable	
Czech/Slovak Republic	3	1	1	1
Greece	9	8	1	
Italy	46	20	14	12
Norway	3	2	1	
Serbia	10	10		
Sweden	3	3		
Turkey	2	2		
Total	76	46	17	13

Molecular identification:
F. verticillioides

two polymorphic genes - the intergenic spacer 1 (IGS1) and the translation elongation factor (TEF) gene

Four children (16 – 19 yrs) BC documented cases of disseminated Fusarium infection in immunocompromised children in Institute for Oncology and Radiology of Serbia (IORS), isolated in Institute of Public Health of

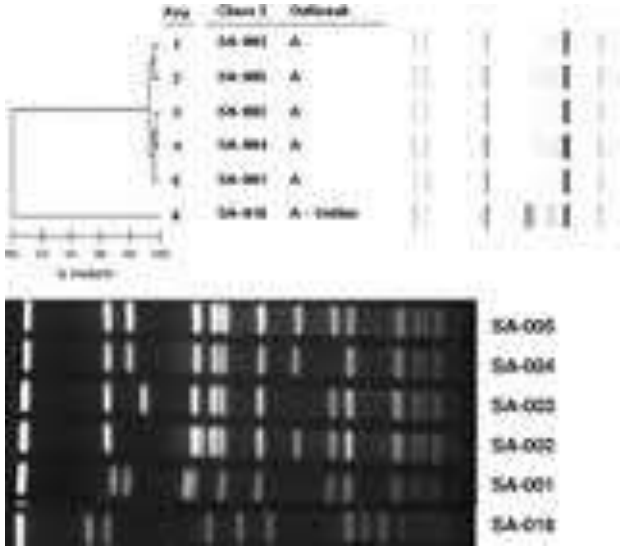
Candida sepsis and nosocomial outbreak in Serbian neonatology settings

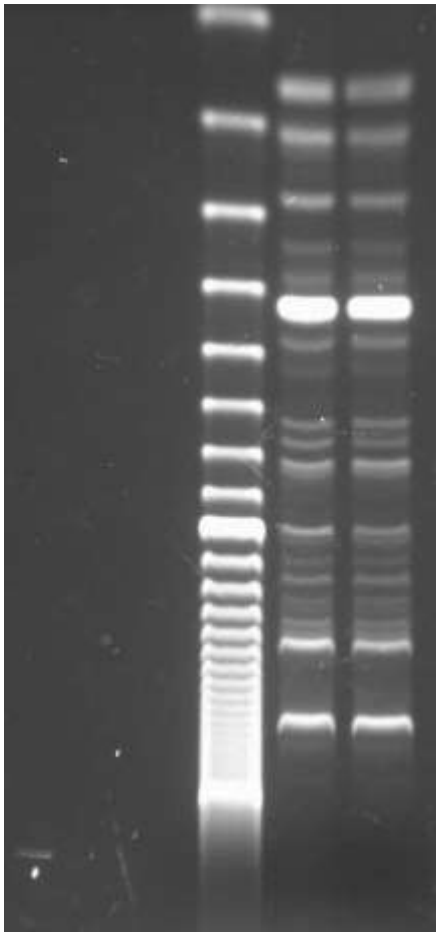
C. albicans fungemia outbreak

in a delivery unit (Belgrade, Serbia) were successful treated with AmB in 11 newborns

Name Date of birth	Maksimović 10.02.09.	Ivanović 19.02.09.	Milojević 24.02.09.	Grujić 24.02.09.	Jockić 26.02.09.	Zorić 28.02.09.	Jovanetić 28.02.09.	Sredanović 28.02.09.	Sredanović 28.02.09.	Krstić 10.03.09.	Novakov 16.05.09.
Risk factors	Gemelli AC, PROM SC	Ab plac part . Asph.perin SC	AC, IUGR PROM SC	VRP 11,20h Vag	VRP 13h Stasis in expuls SC	Gemelli IVF SC	VRP 48h Vag	Gemelli IVF SC	Gemeli IVF SC	Abr plac. Asph.per SC	SC
Gender/ /gestat/ /Apgar score	M 36,1 9/10	M 36,3 9/9	M 35,5 8/8	F 36,1 9/10	M 39,6 7/9	M 36,5 8/9	M 36,4 9/10	F 36,5 9/10	M 36,5 8/10	F 28,3 3/4	F 37,3 8/9
Biomerty g/cm	2850/49	2600/48	1920/46	2400/47	4300/52	2480/48	2800/49	2600/47	2250/46	1020/35	3450/52
Th	L-A-M-V-N	P-A-L-V-N	P-A-L-N- M-V	P-A-O-M	P-A-L-M-N	P-A-L-M-O	P-A	P-A	P-A	P-A	PA
CVK, VUK Cortico.	Inf,VML,Tr FFP	Inf,VML,Tr Ig,FFP, 15.d -CVC	Inf,VML, Tr,Ig	Inf, FFP, Tr,VML 13.d - CVC	Inf, FFP	Inf, FFP, Tr,TrT Ig, VML	Inf	Inf	Inf	Inf	Inf
Microbiology results	**8.d UC:** *Candida*	**16d UC:** *Candida*	**Asp:** *Klebsiela*	**13.d HC:** *Candida* **7.d UC:** *Klebsiela*	**Asp**: *Enterococcus*	**9.d HC:** *Candida*	/	/	/	**HC:** *Candida*	**Asp:** *Candida*
Laboratory results	CRP 115 Le33 Tr 41	CRP 150 Le 4,2 Tr 72	CRP 119 Tr 51	CRP 107 Le 5,1 Tr 53	CRP 56 Le 6,5	CRP 169 Tr 21 Le 6,5	CRP 8,5 Le 9,5	Le 8,0	Le 7,9	/	/
Manifestation - day	7. and 14.	10. and 16.	7.	3. and 7.	7. and 12.	2. and 5.	/	/	/	/	/
ICU/Semi ICU days	SICU-8 ICU-12	ICU 8+8 SICU 2	ICU 13	SICU 7 ICU 9	ICU 7 SICU 15	SICU 1 ICU 11	SICU 7	ICU 6 SICU 4	ICU 6 SICU 4	ICU 1	ICU 1
Infection roure	?	?	?	?	?	?	?	?	?	?	?
Type of infection	Candida sepsis	Candida sepsis ITU	Candida sepsis	Candida sepsis	Candida arthritis septica geni sin	Sy aspir RD Sepsis	?	Left elbow?	?	Asphyxia perinat. RDS	TTN
***Candida spp* Typing**	*C. albicans*	*C. albicans*	*C. albicans*	*C. albicans*	DiversiLab Rep PCR *C. albicans*	*C. albicans*	*C. albicans*	DiversiLab Rep PCR *C. albicans*	*C. albicans*	*C. albicans*	*C. albicans*
Th Candida	Flu/ABCD	Flu/ABCD	Flu/ABCD	Flu/ABCD	Flu/ABCD	Flu/ABCD	Flu/ABCD	Flu/ABCD	Flu/ABCD	Flu/ABCD	Flu/ABCD
Clinical follow up	IMC	IMC	IMC	IMC	IMC	INN	H/UCH	H/UCH	H/UCH	INN	IMC

Rep-PCR strain typing (DiversiLab) *C. albicans* strains isolated from blood of neonates with osteomyelitis





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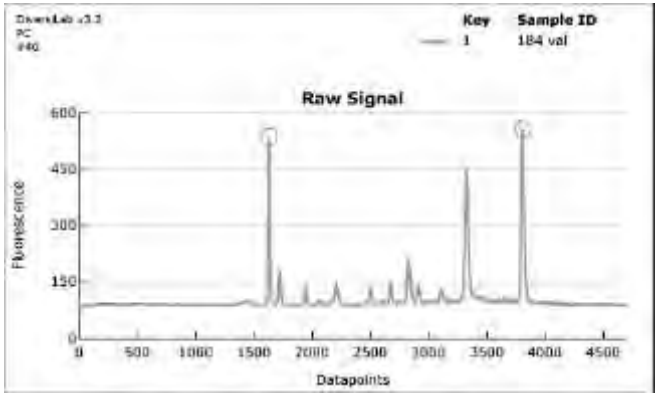
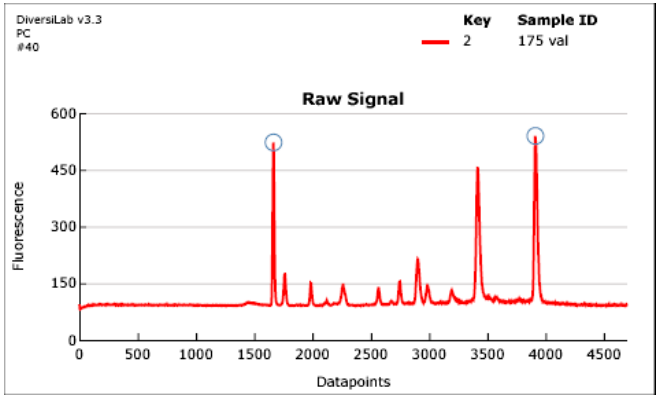
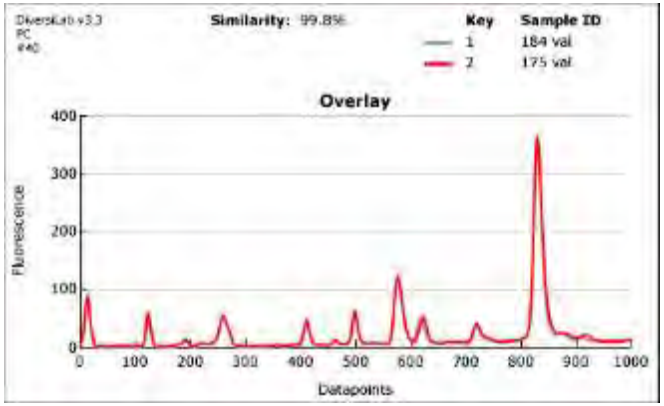
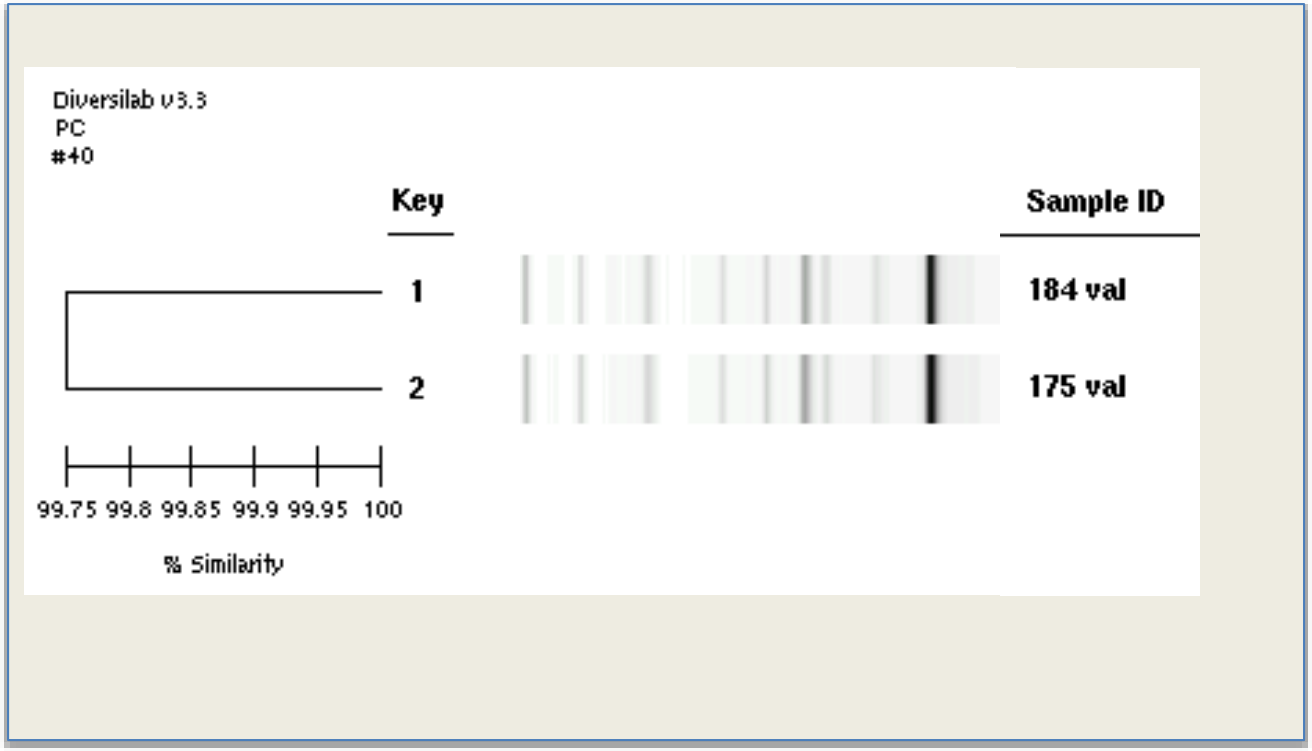
Pulsed-field gel electrophoresis of *Candida albicans* DNA









Genotyping - similarity 99,8%

C.albicans - susceptibility testing *of C. albicans* isolates from different hospitals (No175 and No184)

Conclusions

Blood culture (BC): advantages

(i) they have been in use for more than 100 years and are well integrated in the clinical workflow and clinical guidelines;

(ii) semi-automated BC systems have greatly simplified handling in the microbiological laboratory which results in a short hands-on time;

(iii) a wide range of bacterial and fungal pathogens can be isolated and identified;

(iv) isolation of the pathogen is a prerequisite to phenotypic susceptibility testing which enables clinicians to initiate targeted antimicrobial therapy.

Blood culture (BC): limitations

(i) detection is limited to pathogens that are able to grow in BC and some fungi, such as *Aspergillus* spp., grow poorly;

(ii) antifungals may inhibit growth and relevant pathogens may thus go undetected, after the initiation of antifungalsal therapy;

(iii) BC diagnostics requires one week until results are available and many clinicians feel that results are available too late to guide therapy.

Blood culture (BC): our suceses

(i) Microbiologist with good knowlege in mycology

(ii) For diagnosis: timely collaboration with NR MML

(iii) For treatment: collaboration between clinitians NR MML