Candida haemulonii complex: A Little known candida

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FONDO EUROPEO DE DESARROLLO REGIONAL
"Una manera de hacer Europa"

Abstract 1696

Poster

Background: *Candida haemulonii complex* (*Candida haemulonii* [I], *Candida haemulonii var. Vulnera* [III] and *Candida duobushaemulonii* [II]) has recently acquired relevance, not so much for their high incidence in human clinical sample cultures but for the notable resistance to antifungals. This study aimed to compare several methods of identification of these rare species of *Candida* as well as to evaluate different methods for the study of their antifungal sensitivity.

Material/methods: Between 2011 and 2016, 10 strains of *C. haemulonii complex* have been isolated from clinical samples in our laboratory. They came from blood cultures (4), skin ulcers (2), diabetic foot exudates (2), joint fluid (1) and non-surgical wound exudate (1).
Isolates were analyzed and the results were compared to those of Vitek-MS™ (v3 SARAMIS MS -ID, bioMérieux, Marcy-l´Étoile, France), Vitek2 (bioMérieux, Marcy-l´Étoile, France) and API32C (bioMérieux, Marcy-l´Étoile, France). As reference method the amplification and sequencing of the internal transcribed spacer (ITS) region was used.
Antifungal sensitivity was tested by standardized methods of both CLSI (M27-A3) and EUCAST, and by two comercial methods as Vitek2 (bioMérieux, Marcy-l´Étoile, France) using AST-YS07 cards and Sensititre™ YeastOne YO10 (TREK Diagnostic Systems, East Grinstead, United Kindom).

Results: Sequencing results of the 10 strains identified: 5 *C.haemulonii* [I], 4 *C. duobushaemulonii*[II] and 1 *C. haemulonii var. vulnera*[III]. Both Vitek-MS™ and Vitek2 identified all strains as *C. haemulonii complex* but the first one exhibited a 99% identification percentage while the second showed a lower discrimination with 95.6%. At 24 hours API32C system incorrectly identified i) *C.haemulonii* [I] as *C. globosa*[4] or *C. sake*[1], ii) *C. duobushaemulonii*[II] as *C. sake* and iii) did not identify *C. haemulonii var. vulnera*[III]. At 48 hours the result was the same in 4 strains, did not identify 4 strains and in 2 strains changed the identification to *C. intermedia*.
Results of CLSI method, EUCAST method, Vitek2 and Sensititre™ were similar, and all strains showed resistance to both amphotericin and azoles.

Conclusions: Although both Vitek-MS™ and Vitek2 showed to be valid methods for identifying *C. haemulonii complex*, the former does so with a higher percentage of identification and in less time. None of the methods identified the strains to the species subgroup. There is a good correlation between all methods assayed to perform antifungal susceptibility testing, both with commercial methods and with standardized methods, confirming the resistance of these species to amphotericin β and to the azoles..

Background

Classical identification methods in a clinical microbiology laboratory turn out to be sometimes unreliable. MALDI-TOF MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) has been presented as a fast and reliable method for the identification of bacteria and fungi. *Candida haemulonii complex* (*C. haemulonii* [I], *C. haemulonii var. vulnera* [III] and *C. duobushaemulonii* [II]) have recently acquired relevance, not so much for their high incidence in human clinical sample cultures, but for the notable resistance to antifungals.

Objectives

This study aimed to compare several methods of identification of these uncommon species of *Candida* as well as to evaluate different methods for the study of their antifungal sensitivity.

Material/methods

Between 2011 and 2016, 10 strains of *C. haemulonii complex* have been isolated from clinical samples in our laboratory. They came from blood cultures (4), skin ulcers (2), diabetic foot exudates (2), joint fluid (1) and non-surgical wound exudate (1).
Isolates were analyzed for identification and the results were compared to those of Vitek-MS™ (v3 SARAMIS MS -ID, bioMérieux, Marcy-l´Étoile, France), Vitek2 (bioMérieux, Marcy-l´Étoile, France) and API32C (bioMérieux, Marcy-l´Étoile, France). As reference method the amplification and sequencing of the internal transcribed spacer (ITS) region was used.
Antifungal sensitivity was tested by standardized methods of both CLSI (M27-A3) and EUCAST, and by two comercial methods as Vitek2, using AST-YS07 cards, and Sensititre™ YeastOne YO10 (TREK Diagnostic Systems, East Grinstead, United Kindom).

Results

Strain Number	Maldi-tof MS	Maldi-tof MS %	Vitek2	Vitek2 %	API 20C 24h	API 20C % 24h	API 20C 48h	API 20C % 48h	ITS	ITS GenBank
Strain 1	C.haemulonii	99,99%	C.haemulonii	95%	C. globosa	99,80%	C. globosa	99,80%	C.haemulonii(I)	JX459684.1
Strain 3	C.haemulonii	99,99%	C.haemulonii	98%	C. globosa	99,80%	Not ID	-	C.haemulonii(I)	JX459684.1
Strain 5	C.haemulonii	99,99%	C.haemulonii	96%	C. globosa	99,80%	C. globosa	99,80%	C.haemulonii(I)	JX459773.1
Strain 8	C.haemulonii	99,99%	C.haemulonii	92%	C. globosa	80,30%	C. globosa	99,80%	C.haemulonii(I)	KT968724.1
Strain 10	C.haemulonii	99,99%	C.haemulonii	98%	C. sake	98,90%	C. sake	99,7%	C.haemulonii(I)	JX459664.1
Strain 2	C.haemulonii	99,99%	C.haemulonii	95%	C. sake	87,30%	Not ID	-	C.duobushaemulonii(II)	KU365080.1
Strain 6	C.haemulonii	99,99%	C.haemulonii	98%	C. sake	87,30%	C. intermedia	38,40%	C.duobushaemulonii(II)	KU365080.1
Strain 7	C.haemulonii	99,99%	C.haemulonii	91%	C. sake	80,10%	Not ID	-	C.duobushaemulonii(II)	KU365080.1
strain 9	C.haemulonii	99,99%	C.haemulonii	95%	C. sake	87,30%	C. intermedia	38,40%	C.duobushaemulonii(II)	KU365080.1
Strain 4	C.haemulonii	99,99%	C.haemulonii	98%	Not ID	-	Not ID	-	C.haemulonii var. vulnera(III)	JX459687.1

	Amphotericin B				Fluconazol				5 Flucitosin				Itraconazol				ITS
	Vitek2	Sensititre	CLSI	Eucast	Vitek2	Sensititre	CLSI	Eucast	Vitek2	Sensititre	CLSI	Eucast	Vitek2	Sensititre	CLSI	Eucast	
Strain 1	8	8	4	2	32	>256	>256	>256	<1	0,125	0,06	0,128	NT	1	>16	>16	C.haemulonii(I)
Strain 3	1	4	1	1	32	>256	>256	>256	<1	0,06	0,06	0,128	NT	>16	16	>16	C.haemulonii(I)
Strain 5	8	8	4	2	16	16	>256	>256	<1	0,06	0,06	0,128	NT	>16	>16	>16	C.haemulonii(I)
Strain 8	8	8	4	2	32	>256	>256	>256	<1	0,125	0,06	0,128	NT	16	>16	>16	C.haemulonii(I)
Strain 10	4	4	4	2	16	>256	>256	>256	<1	<0,06	0,06	0,128	NT	>16	>16	>16	C.haemulonii(I)
Strain 2	8	>8	>32	4	>64	>256	32	256	<1	0,5	0,128	0,128	NT	>16	2	16	C.duobushaemulonii(II)
Strain 6	>16	4	4	4	>64	>256	>256	>256	<1	0,125	0,128	0,128	NT	>16	16	>16	C.duobushaemulonii(II)
Strain 7	8	>8	8	2	>64	>256	>256	>256	<1	0,125	0,06	0,128	NT	>16	>16	>16	C.duobushaemulonii(II)
strain 9	2	>8	4	4	16	32	16	128	<1	0,125	0,128	0,128	NT	0,5	1	2	C.duobushaemulonii(II)
Strain 4	8	8	2	1	32	>256	>256	>256	<1	<0,06	0,06	0,128	NT	16	16	>16	C.haemulonii var. vulnera(III)

NT: not tested

Conclusions

Although both Vitek-MS™ and Vitek2 showed to be valid methods for identifying *C. haemulonii complex*, the former exhibited a higher percentage of identification and in less time. However none of the methods identified the strains to the species subgroup. There was a good correlation between all methods assayed to perform antifungal susceptibility testing, both with commercial methods and with standardized methods, confirming the resistance of these species to amphotericin B and to the azoles.

References

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