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Infective endocarditis: an alternative molecular diagnostic approach to improve laboratory analysis of heart valve tissue

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Background

Infective endocarditis (IE) represents a major health challenge. Blood culture-negative infective endocarditis (BCNIE) highlights the limits of traditional culture based methods, resulting in difficulties for diagnosis and treatment. European Society of Cardiology guidelines recommend broad-range 16S/28S PCR with sequencing (1), but this approach is not routinely available in many institutions. This study evaluated the impact of a rapid commercial molecular assay performed on valve samples. This approach was implemented alongside traditional methods as a diagnostic tool for confirming infectious aetiology in IE.

Methods

A retrospective analysis was conducted on 22 patients undergoing cardiac valve replacement. Based on Duke criteria, 12 cases were classified as definite IE, 5 as possible IE and 5 as non-IE. Blood cultures, valve cultures and molecular testing were compared **(Fig. 1).** Both, culture and molecular assays on valve tissue, were performed on the suspension obtained after 0.2% dithiothreitol (DTT) treatment and centrifugation.

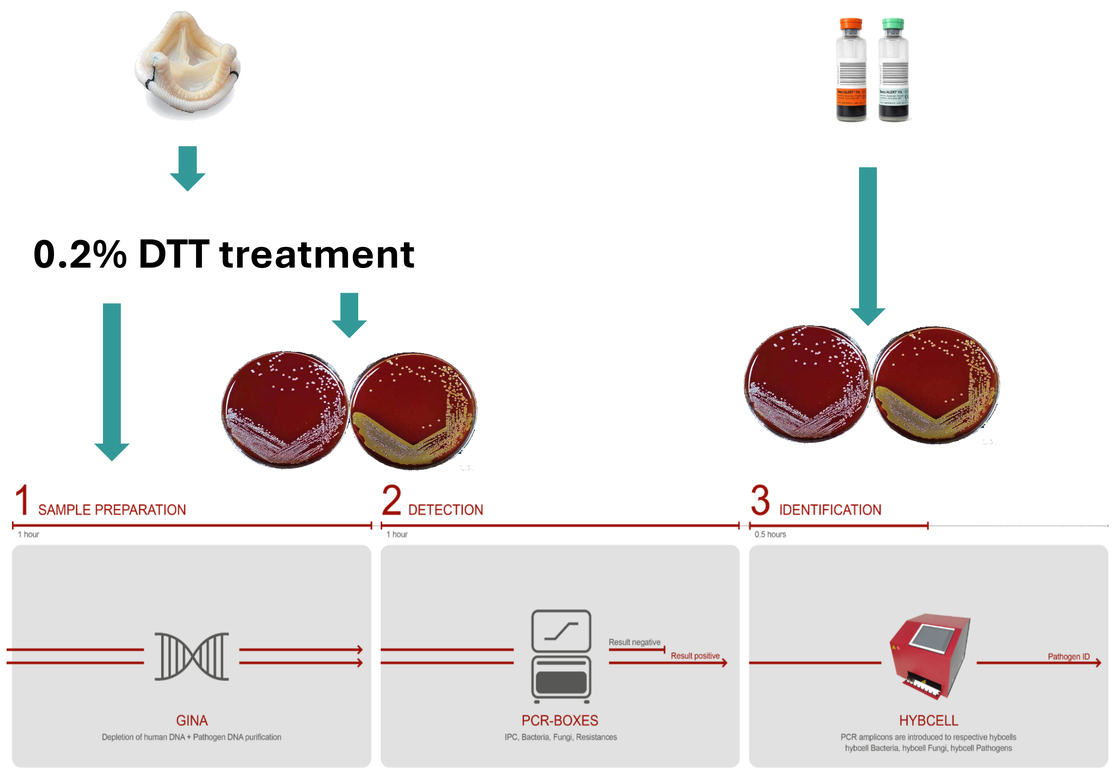

Fig.1 Workflow of the differemt specimen analized with colture-based and molecular method.

Molecular analysis *(off-label)* was performed using Hybcell Bacteria DNA xB®(CubeDx), a 59-target syndromic panel based on a broad-range PCR step, followed by bacterial species identification through microarray hybridization**(Fig.2)**.

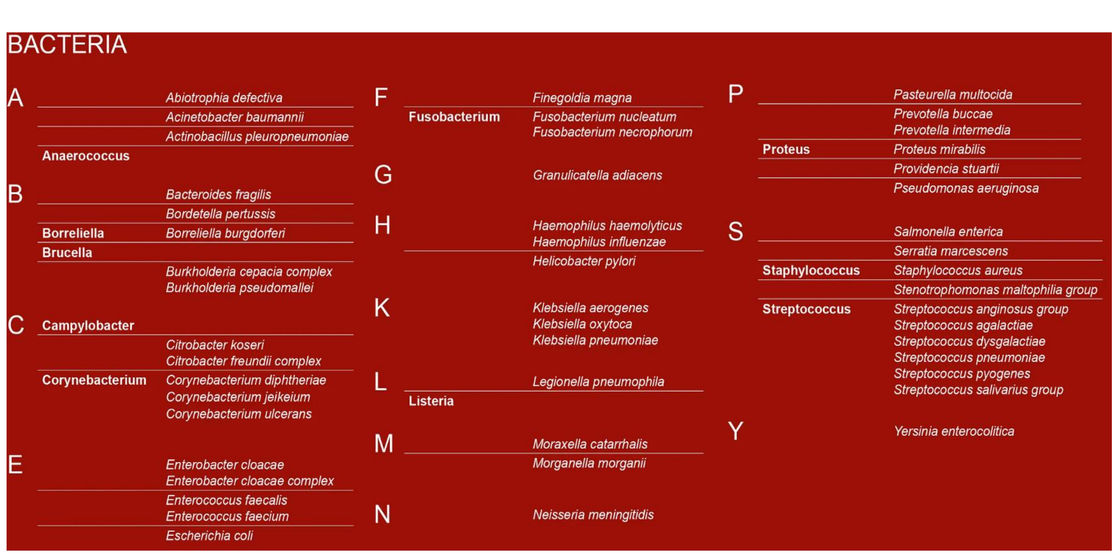

Fig.2 List of bacterial species detectable by Hybcell Bacteria DNA xB (SEPSIS)

Results

The analysis of 17 patients with definite IE or possible IE showed a positivity rate in the valve culture of 23%, whereas blood cultures were positive in 52%. By comparison, the molecular approach demonstrated a higher positivity rate, equal to 70% **(Fig.3).**

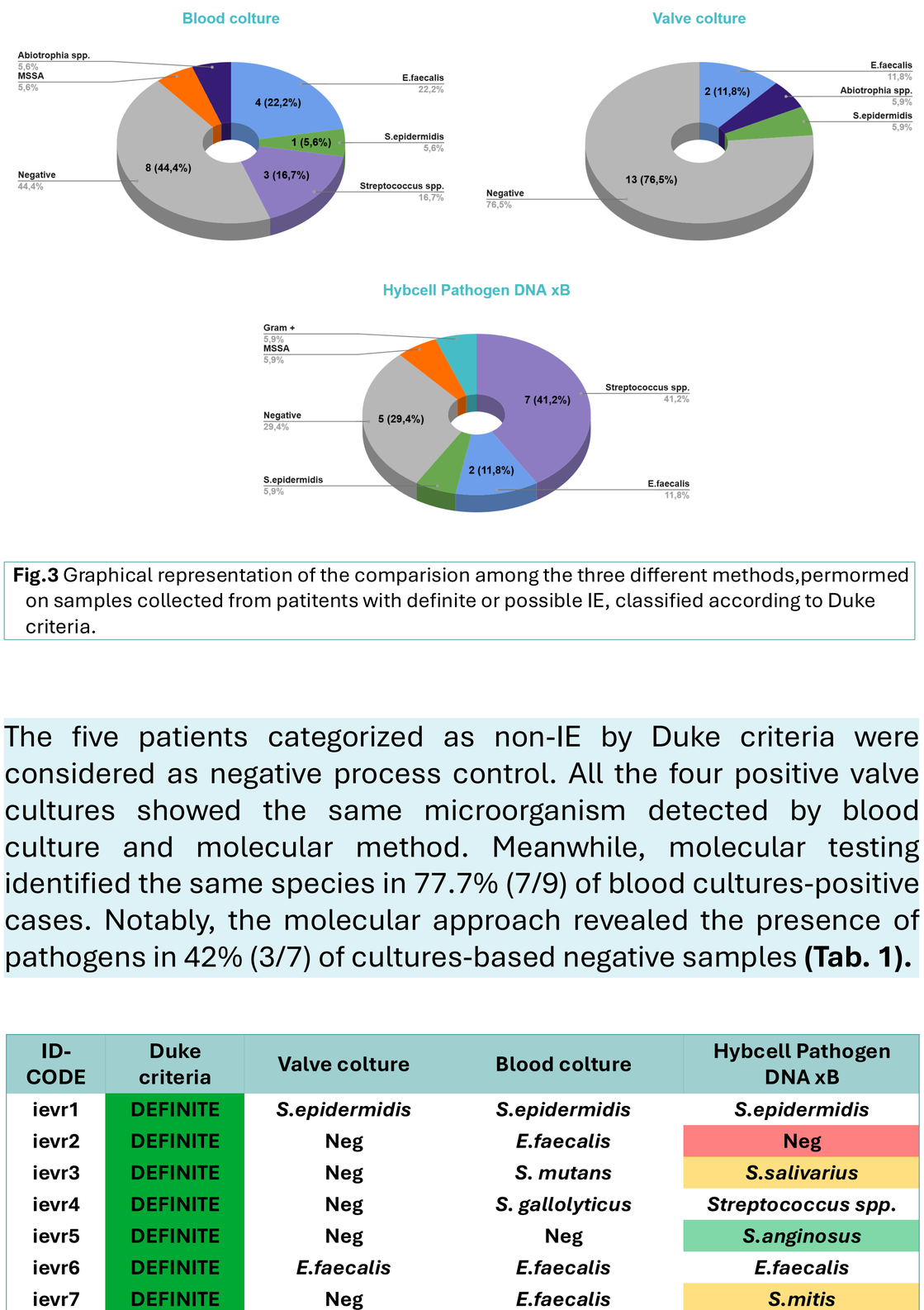

Fig.3 Graphical representation of the comparision among the three different methods,permormed on samples collected from patitents with definite or possible IE, classified according to Duke criteria.

The five patients categorized as non-IE by Duke criteria were considered as negative process control. All the four positive valve cultures showed the same microorganism detected by blood culture and molecular method. Meanwhile, molecular testing identified the same species in 77.7% (7/9) of blood cultures-positive cases. Notably, the molecular approach revealed the presence of pathogens in 42% (3/7) of cultures-based negative samples **(Tab. 1).**

ID-CODE	Duke criteria	Valve colture	Blood colture	Hybcell Pathogen DNA xB
ievr1	DEFINITE	*S.epidermidis*	*S.epidermidis*	*S.epidermidis*
ievr2	DEFINITE	Neg	*E.faecalis*	Neg
ievr3	DEFINITE	Neg	*S. mutans*	*S.salivarius*
ievr4	DEFINITE	Neg	*S. gallolyticus*	*Streptococcus spp.*
ievr5	DEFINITE	Neg	Neg	*S.anginosus*
ievr6	DEFINITE	*E.faecalis*	*E.faecalis*	*E.faecalis*
ievr7	DEFINITE	Neg	*E.faecalis*	*S.mitis*
ievr8	DEFINITE	*Abiotrophia spp.*	*Abiotrophia spp.*	G+
ievr9	DEFINITE	Neg	MSSA	MSSA
ievr10	DEFINITE	*E.faecalis*	*E.faecalis*	*E.faecalis*
ievr11	DEFINITE	Neg	Neg	*Streptococcus spp.*
ievr12	DEFINITE	Neg	*S. gallolyticus*	*Streptococcus spp.*
ievr13	POSSIBLE	Neg	Neg	Neg
ievr14	POSSIBLE	Neg	Neg	Neg
ievr15	POSSIBLE	Neg	Neg	Neg
ievr16	POSSIBLE	Neg	Neg	Neg
ievr17	POSSIBLE	Neg	Neg	*Streptococcus spp.*
ievr18	NON-IE	Neg	Neg	Neg
ievr19	NON-IE	Neg	Neg	Neg
ievr20	NON-IE	Neg	Neg	Neg
ievr21	NON-IE	Neg	Neg	Neg
ievr22	NON-IE	Neg	Neg	Neg

Tab.1 Comparision between colture-based and molceular method. A color-coding system was used to evaluate the agreement: **green** indicates species identified only by Hybcell®, **orange** indicates discordant results and **red** indicated molecular negative but colture positive cases.

Conclusions

This molecular approach significantly improves the detection of pathogens in BCNIE, identifying IE missed by conventional culture methods. In fact, it enhances diagnostic sensitivity, complementing culture and serology-based methods in microbiological IE diagnosis. Furthermore, this method does not require sequencing expertise, making it suitable for settings where broad-range PCR with sequencing is unavailable.

Reference
1. Delgado V, Ajmone Marsan N, de Waha S, et al. 2023 ESC Guidelines for the management of endocarditis. Eur Heart J. 2023;44(39):3948-4042.

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