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Desafios na identificação do *Ochrobactrum anthropi* em hemoculturas e culturas de escarro de pacientes com fibrose cística

Challenges in the identification of Ochrobactrum anthropi in blood and sputum cultures of patients with cystic fibrosis

Desafíos en la identificación del Ochrobactrum anthropi en hemocultivos y cultivos de esputo de pacientes con fibrosis quística

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RESUMO

Justificativa e Objetivos: A identificação do *Ochrobactrum anthropi* é um desafio. Dessa forma, nós realizamos numerosos métodos de identificação em amostras de *O. anthropic* e associamos os resultados obtidos entre os métodos aplicados. **Métodos:** Os seguintes métodos foram realizados: análise fenotípica manual, Vitek®2, Phoenix™, reação em cadeia da polimerase, MALDI-TOF Vitek MS™ e Biotyper™. **Resultados:** Os testes bioquímicos mostraram limitações para identificar *O. anthropic*. O Vitek®2 foi o único método que mostrou o mesmo resultado observado na análise por reação em cadeia da polimerase específica para o *O. anthropic* em todas as amostras, seguido pelo Phoenix™. MALDI-MS™ falhou na definição de espécies, e o MALDI-BD™ apresentou elevada diversidade nos resultados, sendo considerado um teste de baixa especificidade. **Conclusões:** Cada método mostrou vantagens e desvantagens na identificação do *O. anthropic*.

***Descritores**: Bactéria; Diagnóstico; Infecção; Microbiologia; Reação em Cadeia da Polimerase.*

ABSTRACT

Background and Objectives: The *Ochrobactrum anthropic* identification is a challenge. In this context, we performed numerous methods to identify the *O. anthropic* and associated the achieved results among the methods. **Methods:** We performed the follow methods: manual phenotypic, Vitek®2, Phoenix™, polymerase chain reaction, MALDI-TOF Vitek MS™ and BioTyper™. **Results:** Biochemical tests showed limitations to identify *O. anthropic*. Vitek®2 was the only method that showed the same result seen in polymerase chain reaction for *O. anthropic*, in all samples, followed by Phoenix™. MALDI-MS™ failed at defining at species level, and MALDI-BD™ presented high diversity in identification, showing to be a test of low specificity. **Conclusions:** Each method showed advantages and disadvantages to identify the **O. anthropic.**

***Keywords:** Bacteria; Diagnosis; Infection; Microbiology; Polymerase Chain Reaction.*

RESUMEN

Justificación y objetivos: La identificación de *Ochrobactrum anthropi* es un desafío. De esa forma, realizamos numerosos métodos de identificación en muestras de *O. anthropic* y asociamos los resultados obtenidos entre los métodos aplicados. **Métodos:** Se realizaron los siguientes métodos: fenotípico manual, Vitek®2, Phoenix™, reacción en cadena de la polimerasa, MALDI-TOF Vitek MS™ y BioTyper™. **Resultados:** Las pruebas bioquímicas mostraron limitaciones para identificar la *O. anthropic*. Vitek®2 fue el único método que mostró el mismo resultado observado en reacción en cadena de la polimerasa específica para el *O. anthropic* en todas las muestras, seguido de Phoenix™. MALDI-MS™ fracasó en la definición a nivel de especie, y MALDI-BD™ presentó alta diversidad en la identificación, mostrando ser una prueba de baja especificidad. **Conclusiones:** Cada método mostró ventajas y desventajas en la identificación del *O. anthropic*.

***Palabras Clave:** Bacterias; Diagnóstico; Infección; Microbiología; Reacción en Cadena de la Polimerasa.*

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Rare non-fermenting bacteria are a serious epidemiological problem in hospital-acquired infection and cystic fibrosis (CF) because of the lack of knowledge on its existence and the restricted antimicrobial treatment options, which includes knowledge on *Ochrobactrum anthropi*. The genus *Ochrobactrum* are gram negative non-fermenting rods that at present comprises 17 species. The most isolated species in human clinical material are *O. anthropi* and *O. intermedium*. *O. anthropi* formerly was recognized among *Achromobacter* species with characteristic urease positive reaction. *Ochrobactrum* species are closely related to *Brucella* species. *O. anthropi* is oxidase-positive, indole-negative, motility test-positive, trypsin-positive, pyrrolidonyl aminopeptidase (PYR) positive and saccharolytic with rapid acidification of glucose and xylose. Can be distinguished from *O. intermedius* by colistin and netilmicin susceptibility.[1]

Specifically regarding *O. anthropi*, a total of 335 studies are described in the database from the *National Center for Biotechnology Information* in several situations, such as: CF; sepsis; bacteremia due to the catheter contamination in parenteral nutrition; septicemia and pneumonia in preterm, which have been curiously observed in several isolated cases reports, fact associated with rarity of infection by *O. anthropi*.[2-7]

Thus, we conducted a retrospective, cross-sectional, and descriptive study in a tertiary-level hospital, considering the diagnosis *O. anthropi* for different identification methods, namely:

(i) manual phenotypic method: (metabolism in carbohydrate) oxidation and fermentation (OF) of glucose [$C_6H_{12}O_6$], maltose OF [$C_{12}H_{22}O_{11}$], sucrose OF [$C_{12}H_{22}O_{11}$], lactose OF [$C_{12}H_{22}O_{11}$], xylose OF [$C_5H_{10}O_5$]; (Metabolism of amino acids) via Moeller decarboxylase of: lisin [$C_6H_{14}N_2O_2$], ornithine [$C_5H_{12}N_2O_2$], arginine [$C_6H_{14}N_4O_2$], Moeller base control; Simmons citrate agar; aesculin hydrolysis [$C_{15}H_{16}O_9$]; Indole test; tube with broth of 6.5% NaCl; Mannitol broth [$C_6H_{14}O_6$]; tube of jelly; Imipenem disks [$C_{12}H_{17}N_3O_4S$]; disk of Polimixin B [$C_{48}H_{84}N_{16}O_{17}S$]; DNAse test; Pyrrolidonyl arylamidase test (PYR test); oxidase test; disks of ONPG (orto-nitrofenil-β-D-galactosidase, $C_{12}H_{15}NO_8$); motility test; urea test [H_4N_2O];

(ii) automated phenotypic method Vitek®2 (bioMeriéux, Jacarepaguá, Rio de Janeiro, Brazil) and Phoenix™ (Becton, Dickinson and Company, Loverson Circle, Sparks, USA);

(iii) polymerase chain reaction (PCR) (sense 5' – GCA AGC TGG GTG TCG ATC TGG – 3'; antisense 5'– TTC TCG ACG ACA CCG GCC TTT A – 3'). For the PCR method, we used a standard strain by BCCM® LMG (ATCC49687) (Laboratorium voor Microbiologie, Universiteit Ghent, Ledeganckstraat, Ghent, Belgium) as positive control;

(iv) MALDI-TOF Vitek MS (MALDI-MS™) (bioMeriéux, Jacarepaguá, Rio de Janeiro, Brazil) and MALDI-TOF BioTyper™ (MALDI-BD™) (Becton, Dickinson and Company, Loverson Circle, Sparks, USA).

In this study, ten samples (seven of sputum culture in patients with CF and three of blood culture) previously identified as *O. anthropi* by Vitek®2, of high reliability level (of above 95% – very good) were subjected to different methods of bacterial identification. Compared with the result obtained with Phoenix™, there were discrepancies only for one sample, which was identified as *Ralstonia pickettii*.

In a comparative analysis, differently from Vitek®2, all samples were identified as *Ochrobactrum sp* by MALDI-MS™, with scores ranging from 84.1% to 99.9%. In MALDI-BD™, all analyses have had a score superior to two and only four bacteria have been identified as *O. anthropi*, three as *Ochrobactrum tritici*, one as *Ochrobactrum intermedium* and two as *Ochrobactrum sp.*. Briefly, there was a higher diversity of bacteria identified by MALDI-BD™, while MALDI-MS™ showed to be limited at genus level.

In biochemical tests, the identification of five samples such as *O. anthropi,* one as *Burkholderia sp.*, one as *Ralstonia insidiosa,* and in three samples it was not possible to determine species and genus.

Finally, eight samples were evaluated by PCR, determining the final outcome of all samples as *O. anthropi*. Thus, besides the eight samples evaluated by PCR, Vitek®2, Phoenix™, MALDI-BD™, and biochemical tests identified eight, seven, six, and five, respectively, with the same result, namely *O. anthropi*. On the other hand, for MALDI-MS™, there was no sample identified at species level. Also, the major limitation from our work included the absence of 16S rDNA gene sequences use to study the bacterial phylogeny and taxonomy in our data. Data of the carried-out analysis are presented in table 1.

In the study, despite the limiting factor in having two samples in which it was not possible to obtain results using PCR, curiously we found the following results: (i) biochemical tests showed limitations to identify rare gram-negative non-fermenting such as *O. anthropi,* in addition to the identification of other species such as *R. insidiosa* and *Burkholderia sp.*, as well as inconclusive results – which is possibly associated with results reading process, difficulty of interpretation and low prevalence of bacteria; (ii) Vitek®2 is the only method that showed the same result seen in PCR in all samples, followed by Phoenix™ (only one error); (iii) MALDI-MS™ failed at defining at species level, despite showing a high score in the analysis; (iv) MALDI-BD™ presented high diversity in identification, regardless of the small number of samples to be analyzed, showing to be a test of low specificity. Curiously, MALDI-MS™ and MALDI-BD™ might have methodological limitations, mainly associated with the available database, in the identification of rare bacteria.

Therefore, there is need for knowledge on rare bacteria and its identification, including the species *O. anthropi*, since we are still distant from a reality that favors the determining of species in a quick, cheap and effective way, which is of extreme importance in the health field.

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Tabele 1. Comparison between the results using different methods to identify Ochrobactrum anthropi in a reference center.

Origin	Biochemistry	Vitek®2	Conf. Vitek®2	Phoenix™	Conf. Phoenix™	PCR	MALDI-MS™	MALDI-MS™ Score	MALDI-BD™	MALDI-BD™ Score
Cystic fibrosis	*O. anthropi*	*O. anthropi*	Excellent	*O. anthropi*	99%	*O. anthropi*	*Ochrobactrum sp.*	99.9%	*O. anthropi*	2.56
Cystic fibrosis	*O. anthropi*	*O. anthropi*	Excellent	*O. anthropi*	99%	*O. anthropi*	*Ochrobactrum sp.*	99.9%	*O. tritici*	2.40
Cystic fibrosis	Inconclusive	*O. anthropi*	Excellent	*O. anthropi*	99%	N/id	*Ochrobactrum sp.*	99.9%	*O. anthropi*	2.20
Cystic fibrosis	Inconclusive	*O. anthropi*	Excellent	*O. anthropi*	99%	N/id	*Ochrobactrum sp.*	99.9%	*O. intermedium*	2.15
Cystic fibrosis	*O. anthropi*	*O. anthropi*	Excellent	*O. anthropi*	99%	*O. anthropi*	*Ochrobactrum sp.*	87.0%	*O. anthropi*	2.50
Cystic fibrosis	*R. insidiosa*	*O. anthropi*	Excellent	*O. anthropi*	99%	*O. anthropi*	*Ochrobactrum sp.*	87%	*Ochrobactrum* sp	2.33
Cystic fibrosis	*Burkholderia* sp.	*O. anthropi*	Excellent	*O. anthropi*	99%	*O. anthropi*	*Ochrobactrum sp.*	96.4%	*O. anthropi*	2.34
Blood culture	*O. anthropi*	*O. anthropi*	Excellent	*R. pickettii*	90%	*O. anthropi*	*Ochrobactrum sp.*	84.1%	*O. tritici*	2.05
Blood culture	*O. anthropi*	*O. anthropi*	Very good	*O. anthropi*	99%	*O. anthropi*	*Ochrobactrum sp.*	99.9%	*O. tritici*	2.02
Blood culture	Inconclusive	*O. anthropi*	Excellent	*O. anthropi*	99%	*O. anthropi*	*Ochrobactrum sp.*	99.9%	*Ochrobactrum* sp	2.36

PCR, polymerase chain reaction; N/id, non-identified; O. anthropi, Ochrobactrum anthropi; R. pickettii, Ralstonia pickettii; R. insidiosa, Ralstonia insidiosa; O. tritici, Ochrobactrum tritici; O. intermedium; Ochrobactrum intermedium.

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