UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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MODELO EM EXCEL PARA AUXILIAR NA IDENTIFICAÇÃO DE *ENTEROBACTERALES* PARA USUÁRIOS DE PROVAS BIOQUÍMICAS MANUAIS

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Trabalho de conclusão de curso de especialização apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Especialista em Microbiologia Clínica.

Orientador: Prof. Dr. Afonso Luís Barth
Coorientadora: Dr[a] Camila Mörschbächer Wilhem

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RESUMO

Os laboratórios de pequeno e de médio porte que não contam com métodos automatizados fazem a identificação de *Enterobacterales* através de testes bioquímicos manuais. O presente trabalho desenvolveu um modelo em planilha Excel para auxiliar na leitura da identificação bacteriana aos usuários deste perfil de metodologia. A planilha foi também organizada para fornecer as identificações em ordem de probabilidade. Sua validação foi feita através da comparação dos resultados obtidos com os métodos referência e MALDI-TOF MS, tendo como base um total de 38 amostras, das quais 34 (89,5%) apresentaram concordância de resultados com alto índice de probabilidade (superior a 90%). Dos isolados restantes, 04 apresentaram duas possibilidades de identificação bacteriana, o que foi verificado tanto no método clássico como na planilha Excel. Dentre tais amostras, 01 não apresentou o resultado dos testes esperado para sua espécie, enquanto as outras 03 apresentaram resultados de bioquimismo esperados para sua espécie, mas com percentuais abaixo de 90% no índice de probabilidade, possivelmente pelo baixo poder discriminatório dos próprios testes bioquímicos utilizados na rotina para a identificação de alguns gêneros de *Enterobacterales*. Em tais situações, a utilização da planilha Excel proporciona a vantagem de obter os percentuais de ocorrência de cada bactéria e, caso inconclusivos, recomendar a realização de mais testes ou a liberação do resultado apenas por gênero bacteriano. No conjunto de 34 amostras que apresentaram identidade com alto índice de probabilidade, 02 amostras de *Escherichia coli* (amostras 21 e 22) tiveram resultados pouco frequentes para a fermentação da lactose (negativa) e não foram identificadas pela metodologia referência, mas o foram corretamente pela planilha Excel. Em resumo, quando preenchida com os resultados esperados pela literatura, a planilha Excel apontou o microorganismo correto, o que demonstra que eventuais limitações verificadas decorrem de fatores externos a ela. Quando comparados os resultados da planilha Excel com aqueles identificados pelo MALDI-TOF-MS, apenas 04 amostras (todas bactérias do mesmo gênero) apresentaram divergência, sendo identificadas pela tabela como *Klebsiella oxytoca*, enquanto por MALDI-TOF MS foi identificada como *Klebsiella variicola*, microorganismo este isolado recentemente somente pelos métodos genómicos, proteônicos ou moleculares.

Palavras-chave: Enterobacterales; Testes bioquímicos; Excel; MALDI- TOF MS.

ABSTRACT

Small and medium-sized laboratories that do not have automated methods identify *Enterobacterales* through manual biochemical tests. The present work developed a model in an Excel spreadsheet to help users of this methodology profile read the bacterial identification. The worksheet was also organized to provide identifications in order of probability. And its validation was performed by comparing the results obtained with the reference and MALDI-TOF-MS methods, based on a total of 38 isolates, of which 34 (89.5%) showed concordant results with a high probability index (greater than 90%). Of the remaining isolates, 04 presented two possibilities of bacterial identification, which was verified both in the classic method and in the Excel spreadsheet. One of the isolates did not present the expected test results for its species, while the other 03 presented expected biochemechal results for its species, but with percentages below 90% probability index, possibly due to the low discriminatory power of the biochemical tests used routinely to identify some *Enterobacterales* genera. In such situations, the use of an Excel spreadsheet provides the advantage of obtaining the percentages of occurrence of each bacterium and, if inconclusive, recommending further tests or reporting the result only by bacterial genus. In the set of 34 samples that presented identity with a high probability index, 02 samples of *Escherichia coli* (samples 21 and 22) had infrequent results for lactose fermentation (negative) and were not identified by the reference methodology, but were correctly identified by the Excel spreadsheet. In summary, when filled with the results expected by the literature, the Excel spreadsheet identified the correct microorganism, which demonstrates that any limitations that were encounteres were from external factors in relation to the spreadsheet. When comparing the results of the Excel spreadsheet with those indicated by the MALDI-TOF MS, only 04 isolates (all bacteria of the same genus) showed divergence, which were identified by the table as *Klebsiella oxytoca*, while they were identified by MALDI-TOF MS as *Klebsiella variicola*, a microorganism recently isolated only by genomic, proteonic or molecular methods.

Keywords: *Enterobacteriales*; Biochemical tests; Excel; MALDI -TOF MS.

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SUMÁRIO

1 INTRODUÇÃO

Os bacilos Gram negativos pertencentes à família *Enterobacterales* são os isolados mais frequentemente encontrados em espécimes clínicos analisados no setor de microbiologia de um laboratório de análises clínicas. Para sua identificação são utilizados métodos convencionais (manuais), automatizados e, mais atualmente, a espectrometria de massa por ionização/dessorção a laser auxiliada por matriz (MALDI-TOF MS). Os métodos automatizados oferecem muitas vantagens como, por exemplo, a reprodutibilidade e a disponibilidade de fornecer resultados dentro de 24 horas. Além disso, esses métodos apresentam um sistema de interfaceamento que proporciona facilidade ao médico de obter resultados parciais ou integrais do exame de seu paciente (1). As desvantagens, porém, são o custo elevado e a dependência de um sistema fechado, que exige manutenção e renovação de seus softwares (2).

A tecnologia proteômica, com a utilização da metodologia de MALDI-TOF MS, deu início a uma nova era, de modo que novos gêneros foram validados e muitas novas espécies foram descritas (3). No entanto, os custos da implementação da tecnologia de espectrofotometria de massas ainda estão além das possibilidades financeiras de muitos laboratórios.

Os laboratórios de pequeno e de médio porte que não contam com métodos automatizados fazem a identificação de *Enterobacterales* através de testes bioquímicos manuais, nos quais características metabólicas específicas são detectadas nos microrganismos. Após a leitura dos testes, os resultados são interpretados através de consulta em tabelas. Tais tabelas são fornecidas pelas principais literaturas de referência em microbiologia e possuem a caracterização bioquímica dos principais microrganismos, o que permite a identificação da bactéria a partir da coincidência do resultado com o perfil fornecido para espécie/gênero. Com este método, é possível realizar a identificação dos principais gêneros bacterianos de interesse clínico (4) a um custo mais acessível para os laboratórios com menos recursos, embora tal rotina seja problemática em razão da falta de praticidade, principalmente para os profissionais com menor experiência, pois é necessário o manuseio físico de tabelas extensas, demandando demasiado tempo.

É relevante observar que os métodos fenotípicos de identificação microbiana, como os testes bioquímicos manuais, ainda são os métodos de primeira escolha para a realização do diagnóstico microbiológico na maioria dos laboratórios dos países em desenvolvimento (5).

Dados do PNCQ - Programa Nacional de Controle de Qualidade da Sociedade Brasileira de Análises Clínicas, referentes ao ano de 2022, apontam para a mesma realidade: aproximadamente 87% das identificações encaminhadas para o Programa utilizaram métodos manuais, ou seja, foram 23.037 cepas identificadas pelo método manual *versus* 3.405 pelo método automatizado (6).

1.1 OBJETIVOS

1.1.1 Objetivo geral

Elaborar um modelo informatizado em planilha Excel, como alternativa dinâmica de identificação bacteriana para uso em laboratórios de pequeno e médio porte que utilizam os testes bioquímicos para identificação de *Enterobacterales* de forma manual.

1.1.2 Objetivos específicos

a) Desenvolver modelo informatizado em formato de planilha Excel que forneça, automaticamente, após introdução dos resultados encontrados no bioquimismo bacteriano, a identificação do microrganismo em questão;
b) Estruturar um banco de dados através da inserção de tabela com os perfis bioquímicos de cada família/gênero e de tabela com a porcentagem de positividade para cada prova, ambas já determinadas pela literatura especializada;
c) Validar a planilha Excel para identificação bacteriana mediante comparação com os resultados obtidos através do método referência de interpretação, utilizando amostras de isolados clínicos (urina) e cepas provenientes do Controle Externo de Qualidade (PNCQ);
d) Comparar os resultados obtidos pelo método de referência e pelo modelo Excel também com os resultados obtidos por MALDI-TOF MS, utilizando também amostras de isolados clínicos e cepas provenientes do Controle Externo de Qualidade (PNCQ).

3 CONCLUSÃO E PERSPECTIVAS

O modelo informatizado em planilha Excel, atendeu as perpectivas de uma alternativa dinâmica de identificação bacteriana para uso em laboratórios de pequeno e médio porte que utilizam os testes bioquímicos para identificação de *Enterobacterales* de forma manual.

É relevante salientar, acerca da validação realizada, que o Manual de Procedimentos Básicos em Microbiologia Clínica para o Controle de Infecção Hospitar da Agência Nacional de Vigilância Sanitária do Ministério da Saúde, 2002, registra que, qualquer sistema de testes existentes no comércio, com leitura manual ou automatizada, tem limitações no número de provas e de discriminação dos diferentes gêneros e espécies de enterobactérias, de modo que a maioria dos esquemas trabalha com um máximo de 80% de acerto. É importante destacar que nenhum sistema oferece 100% de acerto para a caracterização das espécies de enterobactérias, mas analisam o principal comportamento descrito na literatura (7).

O mesmo Manual também registra que para as espécies dos gêneros *Citrobacter*, *Enterobacter*, *Klebsiella* e *Serratia* os testes mais utilizados apresentam baixo poder de discriminação, sendo a identificação feita pelo maior percentual de probabilidade (7).

Partindo de tais premissas, o exame da validação levada a termo na execução do presente trabalho de finalização de curso permite a conclusão de que a planilha em Excel desenvolvida apresenta índices de conformidade com o método clássico, sobressaindo-se pela maior dinâmica e facilidade de acesso aos resultados, com inegável ganho em termos de otimização das rotinas de trabalho.

Quando comparada com o método MALDI-TOF MS, apresentou discrimimação semelhante dos microrganismos identificados, embora a superioridade do método proteômico em termos de agilidade e acurácia como alternativa para os laboratórios que possuem maior capacidade orçamentária nunca foi alvo de questionamento no presente trabalho.

Após finalizado o processo de desenvolvimento e validação do modelo, o próximo passo tem como objetivo a criação de um aplicativo usando a planilha elaborada neste trabalho como sistema computacional.

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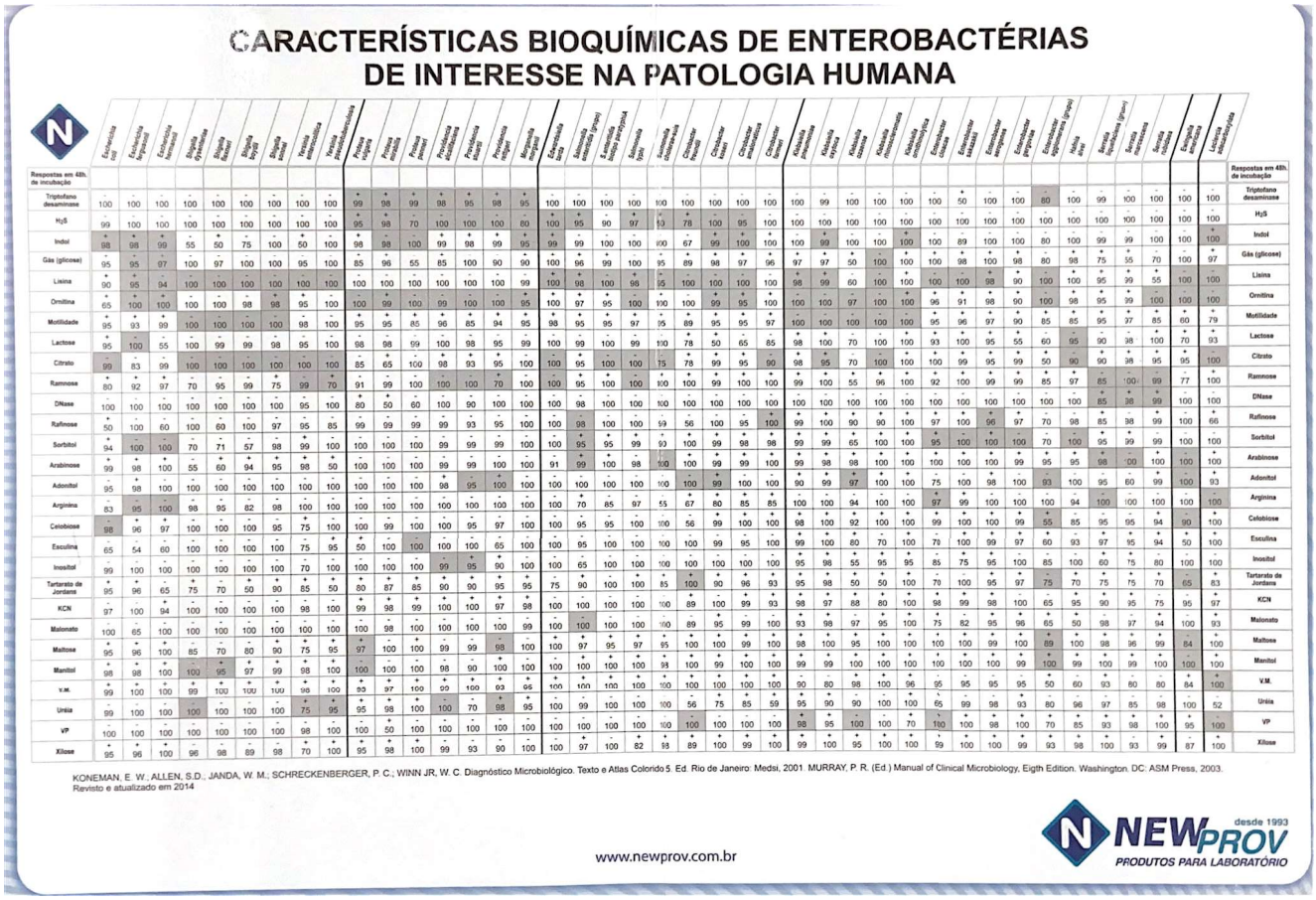
APÊNDICE A – TABELA PERFIL BIOQUÍMICO DAS *ENTEROBACTERALES* DE IMPORTÂNCIA CLÍNICA

BACTÉRIA	GÁS	H2S	URE	IND	MOT	ORN	CIT	LIA	LACT
Escherichia coli	+	-	-	+	+	+	-	+	+
Shigella dysenteriae	-	-	-	-	-	-	-	-	-
Shigella flexneri	-	-	-	+	-	-	-	-	-
Shigella sonnei	-	-	-	-	-	+	-	-	-
Shigella boydi	-	-	-	-	-	-	-	-	-
Salmonella enteritidis (grupo)	+	+	-	-	+	+	+	+	-
Salmonella enteritidis biotipo ***P****aratyphi A*	+	-	-	-	+	+	-	-	-
Salmonella thypi	-	+	-	-	+	-	-	+	-
Salmonella choleraesuis	+	+	-	-	+	+	-	+	-
Citrobacter freundii	+	+	-	-	+	-	+	-	+
Citrobacter koseri (*diversus)*	+	-	+	+	+	+	+	-	+/-
Citrobacter amalonaticus	+	-	+	+	+	+	+	-	-
Klebsiella pneumoniae	+	-	+	-	-	-	+	+	+
Klebsiella oxytoca	+	-	+	+	-	-	+	+	+
Klebsiella aerogenes	+	-	-	-	+	+	+	+	+
Enterobacter cloacae	+	-	+	-	+	+	+	-	+
Enterobacter sakasakii	+	-	-	-	+	+	+	-	+
Enterobacter gergoriae	+	-	+	-	+	+	+	+	+
Enterobacter agglomerans/Grupo	-	-	-	-	+	-	+	-	+/-
Hafnia alvei	+	-	-	-	+	+	-	+	-
Serratia marcescens	+	-	-	-	+	+	+	+	-
Serratia liquefaciens	+	-	-	-	+	+	+	+	-
Proteus vulgaris	+	+	+	+	+	-	-	-	-
Proteus mirabilis	+	+	+	-	+	+	+	-	-
Providencia rettgeri	-	-	+	+	+	-	+	-	-
Providencia stuartii	-	-	-	+	+	-	+	-	-
Morganella morgani	+	-	+	+	+	+	-	-	-
Edwardisiella tarda	+	+	-	+	+	+	-	+	-
Yersinia enterocolitica	-	-	+	+	-	+	-	-	-

APÊNDICE B – TABELA DE POSITIVIDADE DAS *ENTEROBACTERALES* DE IMPORTÂNCIA CLÍNICA FRENTE AOS TESTES BIOQUÍMICOS



CARACTERÍSTICAS BIOQUÍMICAS DE ENTEROBACTÉRIAS DE INTERESSE NA PATOLOGIA HUMANA

Respostas em 48h de incubação	Escherichia coli	Escherichia hermanii	Escherichia vulneris	Shigella dysenteriae	Shigella flexneri	Shigella boydii	Shigella sonnei	Yersinia enterocolitica	Yersinia pseudotuberculosis	Proteus vulgaris	Proteus mirabilis	Proteus penneri	Providencia alcalifaciens	Providencia rettgeri	Providencia stuartii	Morganella morganii	Citrobacter freundii	Salmonella (exceto S. Gallinarum/pullorum)	Salmonella Gallinarum/pullorum	Salmonella typhi	Citrobacter koseri (diversus)	Citrobacter braakii	Citrobacter youngae	Citrobacter amalonaticus	Citrobacter sedlakii	Klebsiella pneumoniae	Klebsiella oxytoca	Klebsiella ozaenae	Klebsiella rhinoscleromatis	Enterobacter cloacae	Enterobacter aerogenes	Enterobacter gergoviae	Enterobacter sakazakii	Enterobacter pyrinus	Enterobacter cancerogenus (group)	Hafnia alvei	Serratia liquefaciens (group)	Serratia marcescens	Serratia rubidaea	Edwardsiella tarda	Edwardsiella hoshinae	Respostas em 48h de incubação
Triptofano desaminase	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Triptofano desaminase
H_2S	100	100	100	100	100	100	100	100	100	95	98	70	100	100	100	80	100	95	90	97	19	78	100	95	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	H_2S
Indol	+	+	+	-	+	-	-	+	-	+	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	Indol
Gás (glicose)	98	98	99	55	50	75	100	50	100	98	98	100	99	98	99	95	99	99	100	100	100	67	99	100	100	100	99	100	100	89	100	100	80	100	99	99	100	100	100	100	100	Gás (glicose)
Lisina	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	-	+	+	+	-	+	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	Lisina
Ornitina	95	95	97	100	97	100	100	95	100	85	96	55	85	100	90	90	100	96	99	100	95	89	98	97	96	97	97	50	100	100	100	98	100	98	80	98	75	55	70	100	97	Ornitina
Motilidade	+	+	+	-	-	-	-	-	-	+	+	-	+	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	Motilidade
Lactose	90	95	94	100	100	100	100	100	100	100	100	100	100	100	100	99	100	98	100	98	95	100	100	100	100	98	99	60	100	100	100	98	90	100	100	100	95	99	55	100	100	Lisina
Citrato	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Ornitina
Ramnose	65	100	100	100	100	98	98	95	100	100	99	100	99	100	100	95	100	97	95	100	100	100	99	95	100	100	100	100	97	100	96	91	98	90	100	98	95	99	100	100	100	Motilidade
DNase	+	+	+	-	-	-	-	-	-	+	+	+	-	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	Lactose
Rafinose	95	93	99	100	100	100	100	98	100	95	95	85	96	85	94	95	98	95	95	97	95	89	95	95	97	100	100	100	100	100	95	96	97	90	85	85	95	97	85	60	79	Motilidade
Sorbitol	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Citrato
Arabinose	95	100	55	100	99	99	98	95	100	98	100	99	98	100	100	95	100	78	50	65	85	78	100	70	100	100	100	93	100	95	55	60	95	90	90	38	100	70	93	100	100	Lactose
Adonitol	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Ramnose
Inositol	99	83	99	100	100	100	100	100	100	85	65	100	98	93	95	100	100	95	100	100	75	78	99	95	90	98	95	70	100	100	100	99	95	99	50	90	90	38	95	95	100	Citrato
Esculina	+	+	+	-	-	-	+	-	+	-	-	-	-	-	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	DNase
Celobiose	80	92	97	70	95	99	75	99	70	91	99	100	100	100	70	100	100	95	100	100	100	100	99	100	100	99	100	55	96	100	92	100	99	99	85	97	85	100	99	77	100	Ramnose
Arginina	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	Celobiose
Lisina	100	100	100	100	100	100	100	95	100	80	50	60	100	90	100	100	100	98	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	85	38	99	100	100	DNase
Malonato	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Rafinose
Manitol	50	100	60	100	60	100	97	95	85	99	99	99	99	93	95	100	100	93	100	100	99	56	100	95	100	99	100	90	90	100	97	100	96	97	70	98	85	38	99	100	66	Sorbitol
Metil-vermelho	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-	-	Arabinose
Voges-Proskauer	94	100	100	70	71	57	98	99	100	100	100	100	99	99	99	100	100	95	95	99	93	100	99	98	98	99	99	65	100	100	95	100	100	100	70	100	95	99	99	100	100	Adonitol
Citrato de Simmons	+	+	+	-	+	+	+	+	+	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	Inositol
Arginina	99	98	100	55	60	94	95	98	50	100	100	100	99	99	100	100	91	99	100	98	100	100	99	99	100	99	98	98	100	100	100	100	99	95	95	98	100	100	100	100	100	Esculina
Uréia	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	Celobiose
Esculina	95	98	100	100	100	100	100	100	100	100	100	100	98	95	100	100	100	70	85	97	55	67	80	85	85	100	100	94	100	100	97	99	100	100	100	94	100	100	100	100	100	Arginina
Gelatinase	98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	Lisina
Fenilalanina desaminase	-	+	+	-	-	-	-	-	-	+	+	+	-	+	+	+	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Malonato
Urease	98	96	97	100	100	100	95	75	100	100	99	100	100	95	97	100	100	95	95	100	100	56	99	100	100	98	100	92	100	100	99	100	100	99	55	85	95	95	94	90	100	Manitol
Descarboxilase Lisina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Metil-vermelho
Descarboxilase Ornitina	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Voges-Proskauer
Descarboxilase Arginina	65	54	60	100	100	100	100	75	95	50	100	100	100	100	65	100	100	95	100	100	100	100	99	95	100	99	100	80	70	100	79	100	99	97	60	93	97	95	94	50	100	Citrato de Simmons
Desaminase Triptofano	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Arginina
Produção de Indol	99	100	100	100	100	100	100	70	100	100	100	100	99	95	90	100	100	65	100	100	100	100	100	100	100	95	98	55	95	95	85	75	95	100	85	100	60	75	80	100	100	Inositol
Produção de Acetoína	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Esculina
Produção de H_2S	+	+	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Tartarato de Jordans
Tartarato de Jordans	95	96	65	75	70	50	90	85	50	80	87	85	90	90	95	95	75	90	100	100	85	100	90	96	93	95	98	50	50	100	70	100	95	97	75	70	75	75	70	65	83	KCN
Utilização do Citrato	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Malonato
Utilização do Malonato	97	100	94	100	100	100	100	98	100	99	98	99	100	100	97	98	100	100	100	100	100	89	100	99	93	98	97	88	80	100	98	99	98	100	65	95	90	95	75	95	97	Maltose
Utilização do Manitol	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Manitol
Utilização do Sorbitol	100	65	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	97	95	97	95	100	100	99	100	99	98	100	100	100	100	100	99	100	89	100	98	96	99	84	100	V.M.
Utilização do Adonitol	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Uréia
Utilização do L-arabinose	98	100	100	100	95	97	99	98	100	100	100	100	98	90	100	100	100	100	100	100	98	100	100	99	100	99	99	100	100	100	100	100	99	100	100	99	100	99	100	100	100	VP
Utilização do Rafinose	99	100	100	100	100	100	100	75	95	95	98	100	99	93	96	100	100	100	100	100	100	100	100	100	100	90	80	98	100	96	95	95	95	95	50	60	93	80	80	84	100	Xilose
Utilização do Xilose	99	100	100	100	100	100	100	75	95	95	98	100	100	100	100	100	100	56	75	85	59	95	90	90	100	100	100	65	99	98	93	80	96	97	85	98	100	52				
Utilização do Xilose	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Utilização do Xilose	95	96	100	96	98	89	98	70	100	95	98	100	99	93	90	100	100	97	100	82	98	89	100	99	100	98	95	100	100	95	100	100	99	93	98	100	93	99	87	100		

KONEMAN, E. W.; ALLEN, S.D.; JANDA, W. M.; SCHRECKENBERGER, P. C.; WINN JR, W. C. Diagnóstico Microbiológico. Texto e Atlas Colorido 5. Ed. Rio de Janeiro: Medsi, 2001. MURRAY, P. R. (Ed.) Manual of Clinical Microbiology, Eigth Edition. Washington, DC: ASM Press, 2003.
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ANEXO A – NORMAS DE PUBLICAÇÃO DO BRAZILIAN JOURNAL OF MICROBIOLOGY

Submission guidelines
Contents
Instructions for Authors
Article types and sections
Scientific style
Manuscript Submission
Title page
Text
References
Tables
Artwork
Supplementary Information (SI)
Research Data Policy and Data Availability Statements
Ethical Responsibilities of Authors
Authorship principles
Competing Interests
Research involving human participants, their data or biological material
Informed consent
Utilization of plants, algae, fungi
Editing Services
After Acceptance
Open Choice
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Mistakes to avoid during manuscript preparation
Instructions for Authors
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Biotechnology and Industrial Microbiology: biosynthesis and bioconversion of natural products, including antibiotics, xenobiotics, and macromolecules produced by bacteria. Biosynthesis and bioconversion of natural products, including antibiotics, xenobiotics, and macromolecules produced by fungi. Molecular aspects of fungal biotechnology. Molecular aspects of bacterial biotechnology.
Food Microbiology: applications of microorganisms (bacteria and fungi) for food production. Food borne diseases, food spoilage, and microbial ecology in foods.
Bacterial and Fungal Pathogenesis: genetic, biochemical, and structural basis of bacterial pathogenesis.
Clinical Microbiology: studies of medically-important bacteria, fungi and virus.
Environmental Microbiology: ecology of natural microbial assemblages, microbial diversity of natural environments such as water, soil, sediments and higher organisms. Microbial interactions. Biodegradation, Bioremediation, and Environmental considerations for genetically engineered microorganisms.
Veterinary Microbiology: diseases of animals, Control and/or treatment of animals, Animal pathogen diagnostics, and Veterinary or zoonotic pathogens.
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Manuscript Submission
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A clear indication and an active e-mail address of the corresponding author
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2. This result was later contradicted by Becker and Seligman [5].
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