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[Continued on next page]

(54) Title: METHODS AND KITS TO IDENTIFY KLEBSIELLA STRAINS

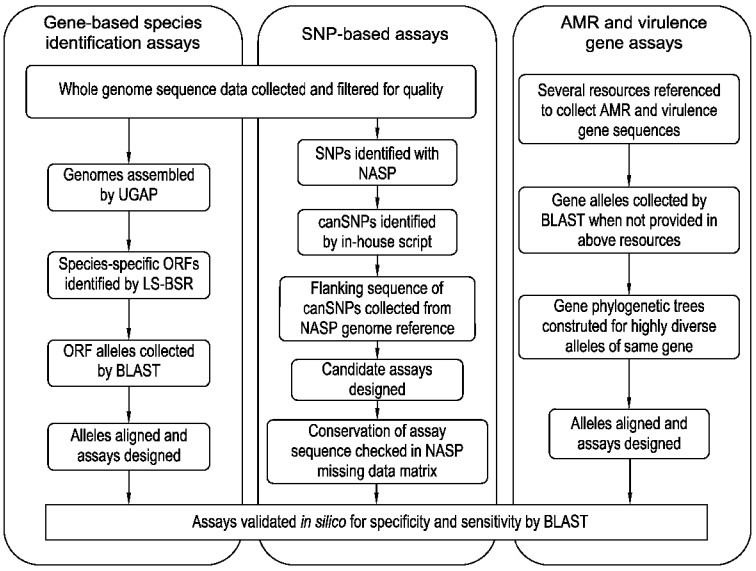

FIG. 1

(57) Abstract: The present invention provides a method of detecting one or more Klebsiella species within a sample from a subject, the method comprising: subjecting DNA and/or RNA from the sample to a PCR amplification reaction using primer pairs targeting species-specific canonical single nucleotide polymorphisms (canSNPs); and analyzing amplification products resulting from the PCR amplification reaction to detect the one or more Klebsiella species. The present invention also provides a kit for detection of one or more Klebsiella species, Klebsiella clonal groups, AMR genes, and/or virulence genes, the kit comprising primer pairs targeting species-specific canSNPs, K. pneumoniae genes M1 and M2, clonal group-specific canSNPs, AMR genes, and/or virulence genes.

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LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12Q 1/68, 1/70; A61P 31/10; C12N 1/14, 1/20, 15/11; C12R 1/01; G06F 19/10 (2017.01)

CPC - C12Q 1/10, 1/6888, 1/689, 1/6895, 1/701, 1/04, 1/68, 1/70; C12N 1/14, 1/20, 15/11; C12R 1/01; G01N 33/48; G06F 19/10; C07H 21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	EP 1 975 253 A1 (INSTITUT PASTEUR) October 01, 2008; paragraphs [0039]-[0064], [0074]; Figure 1c	1, 26 ------- 1-2, 3/1-2
X -- Y	US 2013/0065790 A1 (VOS, P) March 14, 2013; paragraphs [0008], [0050], [0090]-[0097], [0112], [0148], [0160], [0208]-[0220], [0245]	26-27 ------- 1-2, 3/1-2
A	US 2004/0029129 A1 (WANG, L et al.) February 12, 2004; paragraphs [0041], [0795]	28/26-27
A	US 6,610,836 B1 (BRETON, GL et al.) August 26, 2003; column 2, lines 47-48; column 20, lines 1-4	28/26-27
P, X	US 2017/0022543 A1 (THE TRANSLATIONAL GENOMICS RESEARCH INSTITUTE, et al.) January 26, 2017; entire document	1-2, 3/1-2, 26-27, 28/26-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/22211

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/22211

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-25, 29
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Groups I+, Claims 1-3, 26-28; SEQ ID NO:1 (forward primer); SEQ ID NO: 2 (reverse primer)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/22211

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-3, 26-28; SEQ ID NO:1 (forward primer) and SEQ ID NO: 2 (reverse primer) are directed toward a method and kit for detection of one or more Klebsiella species.

The method and kit will be searched to the extent that they encompass SEQ ID NO:1 (first exemplary forward primer) and SEQ ID NO: 2 (first exemplary reverse primer). Applicant is invited to elect additional primer pair(s) with specified pair(s) of SEQ ID NOs: for the forward and reverse primer(s) of each elected pair, to be searched. Additional primer pair(s) will be searched upon the payment of additional fees. It is believed that claims 1-3, 26-27, and 28 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO:1 (forward primer) and SEQ ID NO: 2 (reverse primer). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a primer pair encompassing SEQ ID NO: 3 (first exemplary elected forward primer); SEQ ID NO: 4 (first exemplary elected reverse primer).

No technical features are shared between the primer sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a method of detecting one or more Klebsiella species within a sample from a subject, the method comprising: subjecting DNA and/or RNA from the sample to a PCR amplification reaction using primer pairs targeting species-specific canonical single nucleotide polymorphisms (canSNPs); and analyzing amplification products resulting from the PCR amplification reaction to detect the one or more Klebsiella species; a kit for detection of one or more Klebsiella species, Klebsiella clonal groups, AMR genes, and/or virulence genes, the kit comprising primer pairs targeting species-specific canSNPs, K. pneumoniae genes M1 and M2, clonal group-specific canSNPs, AMR genes, and/or virulence genes.

However, these shared technical features are previously shared by US 2010/0267039 A1 to Driebe, et al. (hereinafter 'Driebe') in view of US 2013/0157876 A1 to The Regents of the University of California (hereinafter 'California').

Driebe discloses a method of detecting one or more within a sample from a subject (a method of detecting one or more within a sample from a subject; abstract; paragraph [0014]), the method comprising: subjecting DNA from the sample to a PCR amplification reaction using primer pairs targeting species-specific canonical single nucleotide polymorphisms (canSNPs) (subjecting DNA and/or RNA from the sample to a PCR amplification reaction using primer pairs targeting species-specific canonical single nucleotide polymorphisms (canSNPs); paragraphs [0030], [0044], [0052]); and analyzing amplification products resulting from the PCR amplification reaction to detect the one or more species (and analyzing amplification products resulting from the PCR amplification reaction to detect the one or more species; Figure 2; paragraphs [0054]-[0058]); a kit for detection of one or more species (claim 71), the kit comprising primer pairs targeting species-specific canSNPs (the kit comprising primer pairs targeting species-specific canSNPs; paragraphs [0030], [0044], [0052]; claim 71).

Driebe does not disclose detecting one or more Klebsiella species.

California discloses detecting one or more Klebsiella species SNPs in a sample (detecting one or more Klebsiella species SNPs in a sample by PCR; paragraphs [0054], [0078], [0135], [0167]; Tables 1, 4).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Driebe, for detecting one or more Klebsiella species, as previously disclosed by California, in order to provide a superior method for identifying additional diagnostic isolates, such as a Klebsiella species, in food and clinical samples.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Driebe and California references, unity of invention is lacking.