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(54) **Title**: PROTEOMICS BASED DIAGNOSTIC DETECTION METHOD FOR CHRONIC SINUSITIS

(57) **Abstract**: The invention provides for a proteomic approach for identification of specific bacterial protein profiles that may be used in the development of methods for the diagnosis of bacterial chronic sinusitis. The invention provides for methods for determining the presence of pathogenic bacteria in the upper respiratory tract of a subject using protein profiles of the pathogenic bacteria. The invention also provides for methods of diagnosing a bacterial infection of the upper respiratory tract of a subject using protein profiles of a pathogenic bacteria. In addition, the invention provides for devices, immunoassays and kits for identifying pathogenic bacteria in the upper respiratory tract.

PROTEOMICS BASED DIAGNOSTIC DETECTION METHOD FOR CHRONIC SINUSITIS

[0001] This invention was made with government support under Grant Nos. R01 DC05847 and KL2RR025754 awarded by the United States National Institutes of Health. The United States government has certain rights in the invention.

[0002] This application claims priority benefit of U.S. Provisional Patent Application No. 61/493,829, filed June 6, 2011, which is incorporated by reference herein in its entirety.

FIELD OF INVENTION

[0003] The invention provides for a proteomic approach for identification of specific bacterial protein profiles that may be used in the development of methods for the diagnosis of bacterial chronic sinusitis. The invention provides for methods of determining the presence for pathogenic bacteria in the upper respiratory tract of a subject using protein profiles of the pathogenic bacteria. The invention also provides for methods of diagnosing a bacterial infection of the upper respiratory tract of a subject using protein profiles of the pathogenic bacteria. In addition, the invention provides for devices, immunoassays and kits for identifying pathogenic bacteria in the upper respiratory tract.

BACKGROUND

[0004] Otitis media, sinusitis, bronchitis, pharyngitis, and nonspecific upper respiratory tract infections (URTI) account for approximately 75% of outpatient antibiotic prescriptions in the United States. Antibiotic use remains high despite the fact that greater than 85% of these infections are due to viruses and resolve without complication. Nonetheless, those remaining infections that are indeed due to bacterial pathogens require more effective management than is currently available. Bacterial cultures provide limited diagnostic value because the most common bacteria responsible for URTI are also often commensal organisms in the nasopharynx.

[0005] Infections of the upper airway are the number one reason for office visits in the US (American Academy of Pediatrics. *Pediatrics,* 2004. 113:1451-1456, Center for Disease Control and Prevention web site, Gonzales R, *et al. JAMA,* 1997. 278(11):901-904, Nyquist A-C. *JAMA*, 1998 . 279(11): 875-877). About 52% of adults patients and 45% of pediatric patients are prescribed antibiotics when diagnosed with an upper airway infection (Gonzales R, *et al. JAMA,* 1997. 278(11):901-904, Nyquist A-C. *JAMA*, 1998 . 279(11): 875-877).

Upper airway infections are multifactorial and polymicrobial diseases. Infection by respiratory viruses (e.g. RSV, adenovirus, rhinovirus, parainfluenza virus) predisposes to bacterial superinfection by members of the nasopharynx normal flora: nontypeable *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*. While viral infections are often self-limiting, therapeutic delay of bacterial disease can lead to complications, permanent sequelae and severe morbidity and mortality.

[0006] Diagnosis is mainly based on clinical manifestations. Signs and symptoms of disease of bacterial and nonbacterial etiologies are often indistinguishable. Specific bacterial identification by traditional microbiological culture techniques often fail to detect microorganisms growing within biofilms. Contamination of specimens by resident colonizing flora often results in laboratory culture reports of uncertain clinical value. Indiscriminate antibiotic use modifies the commensal flora in the nasopharynx and induces the selection and emergence of microorganisms resistant to common antibiotics. Despite a decreasing trend in antibiotic prescription in recent years, unnecessary and inappropriate antibiotic therapies are common, particularly in the treatment of otitis media and sinusitis.

[0007] Upper respiratory tract infection remains as a major cause of overuse of antibiotics and, therefore, a major contributor to the widespread emergence of antibiotic resistance. Therefore, there is a need for early and rapid diagnostic tests that could discriminate between commensal and pathogenic bacteria. These tests would promote judicious use of antibiotic therapy, promote more effective choice of treatment and improve outcomes.

SUMMARY OF INVENTION

[0008] Due to unique growth characteristics, bacterial biofilms produce a distinct set of proteins may be used to distinguish between commensal and pathogenic states. The invention provides for methods of identifying the protein profile of bacterial biofilms. The methodology involves detecting trace quantities of signature proteins that identify specific bacterial pathogens from typically sterile sites in the paranasal sinus cavities. As described herein, biofilms produced by nontypeable *Haemophilus influenzae* (NTHI) over 10 days generate a specific protein profile. Biofilms formed by NTHI *in vitro* release a signature set of proteins into their environment that remains identifiable for several days. Outer membrane proteins (OMPs) are predominant components of the NTHI biofilm supernatant. Of particular interest are major OMPs associated with bacterial virulence: outer membrane protein P5 (OMP P5) and outer membrane protein P2 (OMP P2). Additional OMPs include

high molecular weight adhesin 1/high molecular weight adhesin 2 (HMW1/HMW2), and IgA-protease. HMW1/HMW2, OMP P5 are mediators of adhesion to epithelial cells, OMP P2 is a porin and IgA protease functions to cleave host IgA.

[0009] These studies support the development of a clinical diagnostic test and device for early and rapid identification of NTHI -associated URTIs, leading to a more effective choice of treatment and improved outcomes. NTHI was used as an example for the study but the same methods may be used to identify the presence of any pathogenic bacteria including those known to cause chronic sinusitis such as *Haemophiius influenza, Streptococcus pneumoniae*, *Moraxella catarrhalis, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*.

[0010] The invention also provides for an immunoassay device that involves obtaining a sample of the secretions within the typically sterile paranasal sinus cavities, and rapidly detecting the presence of trace quantities of signature proteins that identify specific bacterial pathogens from these typically sterile sites.

[0011] The invention provides for methods of detecting the presence of a pathogenic bacteria in the upper respiratory tract of a subject comprising the steps of: a) obtaining a sample of secretions from the upper respiratory tract of the subject; b) generating a protein profile of the sample; c) comparing the protein profile with a reference protein profile, wherein the reference protein profile identifies a pathogenic bacteria; and d) determining whether the protein profile of the sample associates to the reference protein profile, wherein association is indicative of the presence of the pathogenic bacteria in the upper respiratory tract of the subject.

[0012] The invention also provides for methods of detecting the presence of a pathogenic bacteria in the upper respiratory tract of a subject as described above wherein the method further comprises the step of administering a therapeutic compound to reduce or eliminate the pathogenic bacteria in the upper respiratory tract of the subject. Exemplary therapeutic compounds that reduce or eliminate pathogenic bacteria in the upper respiratory tract include antibiotics such as penicillin, erythromycin, amoxicillin, thimethoprim-sulfamethoxazole, doxycyline, cefpodoxime, cefuroxime, cefdinir, clarithromycin, azithromycin, levofloxacin, gatifloxacin, and moxifloxacin, alpha-adreneric agonists such as oxymetazoline hydrochloride, anticholinergic (parasympatholytic) agents such as ipratropium bromide, antihistamines such as chlorpheniramine maleate, beta-agonist bronchodilators, non-steroidal

anti-inflammatory drugs, camphor, menthol, Echinacea, mast cell stabilizers such as cromolyn sodium, topical nasal steroids such as fluticasone propionate and zinc salts.

[0013] The invention also provides for methods of detecting the presence of a pathogenic bacteria in the upper respiratory tract of a subject as described above wherein the method further comprises the step of informing the subject of the presence or absence of the pathogenic bacteria in the upper respiratory tract.

[0014] The invention also provides for methods of detecting the presence of a pathogenic bacteria in the upper respiratory tract of a subject as described above wherein the method further comprises the step of diagnosing the subject with a bacterial infection, wherein the presence of the pathogenic bacteria in the upper respiratory tract of the subject is indicative of a bacterial infection.

[0015] The term "pathogenic bacteria" refers to any disease causing bacteria. The term "commensal bacteria" refers to harmless or non-disease causing bacteria. The methods of the invention also may be used to distinguish the presence of commensal bacteria verses pathogenic bacteria in the upper respiratory tract of a subject.

[0016] The invention also provides for methods of diagnosing a bacterial infection in the upper respiratory tract of a subject comprising the steps of: a) obtaining a sample of secretions from the upper respiratory tract of the subject; b) generating a protein profile of the sample; c) comparing the protein profile of the sample with a reference protein profile, wherein the reference protein profile indentifies a pathogenic bacteria; and d) determining whether the protein profile of the sample associates to the protein profile; wherein association is indicative of a bacterial infection in the upper respiratory tract of the subject.

[0017] The invention also provides for methods of diagnosing a bacterial infection in the upper respiratory tract of a subject as described above wherein the method further comprises the step of the step of informing the subject of the diagnosis of a bacterial infection in the upper respiratory tract.

[0018] The invention also provides for methods of diagnosing a bacterial infection in the upper respiratory tract of a subject as described above wherein the method further comprises the step of administering a therapeutic compound to treat the bacterial infection. A treatment for a bacterial infection will reduce or alleviate the symptoms caused by the pathogenic bacteria or eliminate the bacteria from the site of infection. Exemplary therapeutic compounds that treat a bacterial infection in the upper respiratory tract include antibiotics

such as penicillin, erythromycin, amoxicillin, thimethoprim-sulfamethoxazole, doxycyline, cefpodoxime, cefuroxime, cefdinir, clarithromycin, azithromycin, levofloxacin, gatifloxacin, and moxifloxacin, alpha-adreneric agonists such as oxymetazoline hydrochloride, anticholinergic (parasympatholytic) agents such as ipratropium bromide, antihistamines such as chlorpheniramine maleate, beta-agonist bronchodilators, non-steroidal anti-inflammatory drugs, camphor, menthol, Echinacea, mast cell stabilizers such as cromolyn sodium, topical nasal steroids such as fluticasone propionate, budenoside, mometasone, triamcinolone, and dexamethasone, and zinc salts. The term "protein profile" refers to at least one protein that is at least partially identified or characterized so that the presence or absence of the protein in any particular sample may be monitored. The term "reference protein profile" refers to a protein profile generated for a known control or standard sample.

[0019] A protein profile of a sample associates with a reference protein profile when one or more the proteins in the reference profile are present in the sample profile at a concentration that indicates infection or pathogenicity of the bacteria. To determine if a sample protein profile associates with a reference protein profile, the profiles are scored to predict how likely the mass of a fragment that it detected is likely from the peptide sequence it is predicted it to be, and how much quantity of the peptide there is in the supernatant. Software programs that analyze mass spectrometry data may be used. For example, Mascot (Matrix Science, Boston, MA), performs mass spectrometry data analysis through a statistical evaluation of matches between observed and projected peptide fragments rather than cross correlation may be used to determine in the sample associates with a reference protein profile. See, e.g., Electrophoresis, **20(18)** 3551-67 (1999).

[0020] The preceding methods may be carried out for any pathogenic bacteria which infects the upper respiratory tract, including *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* or *Stenotrophomonas maltophilia*.

[0021] The invention also provides for uses of a therapeutic compound for the preparation of a medicament to reduce or eliminate the pathogenic bacteria in the upper respiratory tract of a subject or uses to treat a bacterial infection in the upper respiratory tract of a subject, wherein the subject has a protein profile that associates to a reference protein profile, and wherein the association is indicative of the presence of the pathogenic bacteria or bacterial infection in the upper respiratory tract of the subject as determined by any of the preceding

methods of detecting the presence of a pathogenic bacteria or diagnosing a bacterial infection in the upper respiratory tract of a subject.

[0022] The invention also provides for therapeutic compositions for the reduction or elimination of a pathogenic bacteria in the upper respiratory tract of a subject or for the treatment of a bacterial infection in the upper respiratory tract of a subject, wherein the subject has a protein profile that associates to a reference protein profile, and wherein the association is indicative of the presence of the pathogenic bacteria or bacterial infection in the upper respiratory tract of the subject tract of the subject as determined by any of the preceding methods of detecting the presence of a pathogenic bacteria or diagnosing a bacterial infection in the upper respiratory tract of a subject.

[0023] Exemplary therapeutic compounds that treat a bacterial infection in the upper respiratory tract include antibiotics such as penicillin, erythromycin, amoxicillin, thimethoprim-sulfamethoxazole, doxycyline, cefpodoxime, cefuroxime, cefdinir, clarithromycin, azithromycin, levofloxacin, gatifloxacin, and moxifloxacin, alpha-adreneric agonists such as oxymetazoline hydrochloride, anticholinergic (parasympatholytic) agents such as ipratropium bromide, antihistamines such as chlorpheniramine maleate, beta-agonist bronchodilators, non-steroidal anti-inflammatory drugs, camphor, menthol, Echinacea, mast cell stabilizers such as cromolyn sodium, topical nasal steroids such as fluticasone propionate, budenoside, mometasone, triamcinolone, and dexamethasone, and zinc salts.

[0024] In another aspect of the invention, the invention provides for methods of detecting the presence of Nontypeable *Haemophilus influenzae* (NTHI) bacteria in the upper respiratory tract of a subject comprising the steps of: a) obtaining a sample from the upper respiratory tract of the subject; b) detecting the presence of at least one biomarker in the sample, wherein the biomarkers are selected from the group consisting of: HMW1/HMW2, OMP P5 , OMP P2 and IgA-protease, and wherein the presence of at least one biomarker indicates the presence of NTHI bacteria in the upper respiratory tract of the subject. In one embodiment, the method comprises detecting the presence of OMP P2 and/or OMP P5 in the sample, wherein the presence of OMP P2 and/or OMP P5 indicates the presence of NTHI bacteria in the upper respiratory tract of the subject.

[0025] The invention also provides for methods of detecting the presence of NTHI bacteria in the upper respiratory tract of a subject wherein the method further comprises the step of administering a therapeutic compound to reduce or eliminate the NTHI bacteria in the upper respiratory tract of the subject. Exemplary therapeutic compounds that reduce or eliminate

the NTHI bacteria in the upper respiratory tract include antibiotics such as penicillin, erythromycin, amoxicillin, thimethoprim-sulfamethoxazole, doxycyline, cefpodoxime, cefuroxime, cefdinir, clarithromycin, azithromycin, levofloxacin, gatifloxacin, and moxifloxacin, alpha-adreneric agonists such as oxymetazoline hydrochloride, anticholinergic (parasympatholytic) agents such as ipratropium bromide, antihistamines such as chlorpheniramine maleate, beta-agonist bronchodilators, non-steroidal anti-inflammatory drugs, camphor, menthol, Echinacea, mast cell stabilizers such as cromolyn sodium, topical nasal steroids such as fluticasone propionate, budenoside, mometasone, triamcinolone, and dexamethasone, and zinc salts.

[0026] The invention also provides for methods of diagnosing a NTHI infection in the upper respiratory tract of a subject comprising the steps of: a) obtaining a sample of secretions from the upper respiratory tract of the subject, b) detecting the presence of at least one biomarker in the sample, wherein the biomarkers are selected from the group consisting of: HMW1/HMW2, OMP P5, OMP P2 and IgA-protease, and wherein the presence of at least one biomarkers indicates an NTHI infection in the upper respiratory tract of the subject. In one embodiment, the method comprises detecting the presence of OMP P2 and/or OMP P5 in the sample, wherein the presence of OMP P2 and/or OMP P5 indicates a NTHI bacterial infection in the upper respiratory tract of the subject.

[0027] The invention also provides for methods of diagnosing a NTHI infection in the upper respiratory tract of a subject as described above wherein the method further comprises the step of informing the subject of the diagnosis of a NTHI infection in the upper respiratory tract.

[0028] The invention also provides for methods of diagnosing NTHI infection in the upper respiratory tract of a subject as described above wherein the method further comprises the step of administering a therapeutic compound to treat the NTHI infection in the upper respiratory tract of the subject. A treatment for a NTHI infection will reduce or alleviate the symptoms caused by the NTHI bacteria or eliminate the NTHI bacteria from the site of infection. Exemplary therapeutic compounds that treat a NTHI infection in the upper respiratory tract include antibiotics such as penicillin, erythromycin, amoxicillin, thimethoprim-sulfamethoxazole, doxycyline, cefpodoxime, cefuroxime, cefdinir, clarithromycin, azithromycin, levofloxacin, gatifloxacin, and moxifloxacin, alpha-adreneric agonists such as oxymetazoline hydrochloride, anticholinergic (parasympatholytic) agents such as ipratropium bromide, antihistamines such as chlorpheniramine maleate, beta-agonist

bronchodilators, non- steroidal anti-inflammatory drugs, camphor, menthol, Echinacea, mast cell stabilizers such as cromolyn sodium, topical nasal steroids such as fluticasone propionate, budenoside, mometasone, triamcinolone, and dexamethasone, and zinc salts.

[0029] The term "upper respiratory tract" includes the nose or nostrils, nasal cavity, mouth, throat (pharynx), paranasal sinus cavity and voice box (larynx). The respiratory system is lined with a mucous membrane that secretes mucus or fluid. This secreted mucus and fluid is referred to herein as "secretions." In any of the preceding methods, the sample of secretions may be collected from the paranasal sinus cavity including the middle meatus or the ethmoid infundibulum. The "paranasal sinus cavity" refers to the frontal sinuses (in the forehead), maxillary sinuses (behind the cheek bones), ethmoid sinuses (between the eyes) and the sphenoid sinuses (behind the eyes).

[0030] The invention also provides for use of a therapeutic compound for the preparation of a medicament to reduce or eliminate NTHI bacteria in the upper respiratory tract of a subject or to treat a NTHi infection in the upper respiratory tract of a subject, wherein the presence of NTHI bacteria or a NTHI infection is determined by the presence of at least one biomarker selected from OMP P2 and OMP P5 as determined by any of the preceding methods of detecting the presence of a NTHI bacterial or diagnosing a NTHI infection in the upper respiratory tract of a subject method as determined by the preceding methods of detecting the presence of NTHI bacteria or diagnosing a NTHI infection in the upper respiratory tract of a subject.

[0031] The invention also provides for a therapeutic composition for the reduction or elimination of NTHI bacteria or for the treatment of NTHI infection in the upper respiratory tract of a subject, wherein the presence of NTHi bacteria or NTHI infection as determined by any of the preceding methods of detecting the presence of a NTHI bacterial or diagnosing a NTHI infection in the upper respiratory tract of a subject.

[0032] Any of the preceding methods, uses or therapeutic compositions may be carried out on a subject suffering from chronic sinusitis, or a subject that is prone to suffering from recurrent acute sinusitis. In addition, any of the preceding methods may be carried out on a subject suffering from Otitis media, bronchitis, pharyngitis, and nonspecific upper respiratory tract infections.

[0033] The invention also provides for methods, uses or therapeutic compositions for treating chronic sinusitis or a pathogenic bacterial infection of the upper respiratory tract in a

subject comprising detecting a pathogenic bacteria in the upper respiratory tract of the subject using any of the preceding methods and administering the appropriate dose of a therapeutic compound known to effectively treat the particular pathogenic bacteria detected within the upper respiratory tract of the subject. A treatment for a chronic sinusitis or a pathogenic bacterial infection will reduce or alleviate the symptoms caused by the pathogenic bacteria or eliminate the pathogenic bacteria from the site of the infection. Exemplary therapeutic compounds include antibiotics such as penicillin, erythromycin, amoxicillin, thimethoprim-sulfamethoxazole, doxycyline, cefpodoxime, cefuroxime, cefdinir, clarithromycin, azithromycin, levofloxacin, gatifloxacin, and moxifloxacin, alpha-adreneric agonists such as oxymetazoline hydrochloride, anticholinergic (parasympatholytic) agents such as ipratropium bromide, antihistamines such as chlorpheniramine maleate, beta-agonist bronchodilators, non-steroidal anti-inflammatory drugs, camphor, menthol, Echinacea, mast cell stabilizers such as cromolyn sodium, topical nasal steroids such as fluticasone propionate, budenoside, mometasone, triamcinolone, and dexamethasone, and zinc salts.

[0034] The invention also provides for methods of treating, uses and therapeutic compositions for chronic sinusitis or a pathogenic bacterial infection of the upper respiratory tract in a subject comprising diagnosing a pathogenic bacteria infection in the upper respiratory tract of the subject using any of the preceding methods and administering the appropriate dose of a therapeutic compound known to effectively treat the particular pathogenic bacteria detected within the upper respiratory tract of the subject. Exemplary therapeutic compounds include antibiotics such as penicillin, erythromycin, amoxicillin, thimethoprim-sulfamethoxazole, doxycyline, cefpodoxime, cefuroxime, cefdinir, clarithromycin, azithromycin, levofloxacin, gatifloxacin, and moxifloxacin, alpha-adreneric agonists such as oxymetazoline hydrochloride, anticholinergic (parasympatholytic) agents such as ipratropium bromide, antihistamines such as chlorpheniramine maleate, beta-agonist bronchodilators, non-steroidal anti-inflammatory drugs, camphor, menthol, Echinacea, mast cell stabilizers such as cromolyn sodium, topical nasal steroids such as fluticasone propionate, budenoside, mometasone, triamcinolone, and dexamethasone and zinc salts.

[0035] In any of the preceding methods, uses or therapeutic compositions of the invention, the sample may be collected using sterile swabs, sterile gauze, nasal washing, suction tube or a balloon catheter.

[0036] For the detecting step in any of the preceding methods of the invention, the biomarker may be detected using a monoclonal antibody. In addition, an immunoassay may be used to detect the biomarker of interest in any of the preceding methods of the invention.

[0037] In any of the preceding methods of the invention, the sample may be collected with a device comprising a substrate presenting antibodies specific for the biomarkers of interest, such as a balloon catheter wherein the substrate is threaded into the suction port of the catheter.

[0038] An another aspect of the invention provides for immunoassays for detecting the presence of a pathogenic bacteria in the upper respiratory tract of a subject comprising the steps of a) obtaining a sample of secretions from the upper respiratory tract of the subject using a device comprising antibodies specific for at least one biomarker associated with the presence of a pathogenic bacteria in the upper respiratory tract of the subject; b) detecting the presence of at least one biomarker associated with the presence of a pathogenic bacteria in the upper respiratory tract of the subject to generate a protein profile; c) comparing the protein profile with a reference protein profile, wherein the reference protein profile identifies a pathogenic bacteria; and d) determining whether the protein profile of the sample associates to the reference protein profile, wherein association is indicative of the presence of the pathogenic bacteria in the upper respiratory tract of the subject.

[0039] The term "immunoassay" is a laboratory approach to directly or indirectly detect protein or peptide in fluid, e.g. biological fluid, by use of an immunological reaction between an antigen and an antibody.

[0040] The term "antibody" is synonymous with "immunoglobulin," and includes naturally occurring human antibodies, polyclonal antibodies, and monoclonal antibodies. The term "antibody" is meant to include both the native antibody and biologically active and synthetic derivatives of antibodies, such as, for example, Fab', $F(ab'')_2$ or Fv as well as single-domain and single-chain antibodies. A biologically active derivative of an antibody retains the ability to bind an antigen. In particular, the invention provides for methods and immunoassays that use antibodies specific for the biomarkers of interests, such as monoclonal antibodies that specifically bind biomarkers of interest, *e.g.* OMP P2 and OMP P5.

[0041] In addition, the immunoassays described above may further comprising a step of diagnosing the subject with a bacterial infection wherein the presence of the pathogenic bacteria in the upper respiratory tract of the subject is indicative of a bacterial infection.

[0042] The invention also provides for any of the preceding immunoassay further comprising the step of administering a therapeutic compound in an amount effective to treat the bacterial infection. Exemplary therapeutic compounds include antibiotics such as penicillin, erythromycin, amoxicillin, thimethoprim-sulfamethoxazole, doxycyline, cefpodoxime, cefuroxime, cefdinir, clarithromycin, azithromycin, levofloxacin, gatifloxacin, and moxifloxacin, alpha-adreneric agonists such as oxymetazoline hydrochloride, anticholinergic (parasympatholytic) agents such as ipratropium bromide, antihistamines such as chlorpheniramine maleate, beta-agonist bronchodilators, non-steroidal anti-inflammatory drugs, camphor, menthol, Echinacea, mast cell stabilizers such as cromolyn sodium, topical nasal steroids such as fluticasone propionate, budenoside, mometasone, triamcinolone, and dexamethasone, and zinc salts.

[0043] In any of the preceding immunoassays, the pathogenic bacteria detected may be *Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, Pseudomonas aeruginosa* or *Stenotrophomonas maltophilia*.

[0044] The invention also provides for uses of a therapeutic compound for the preparation of a medicament to reduce or eliminate NTHI bacteria in the upper respiratory tract of a subject or to treat a NTHi infection in the upper respiratory tract of a subject, wherein the presence of NTHi bacterial or a NTHi infection is determined by the presence of at least one biomarker selected from OMP P2 and OMP P5 as determined by any of the preceding

[0045] In addition, the invention provides for a therapeutic composition for the reduction or elimination of NTHI bacteria in the upper respiratory tract of a subject or for the treatment of NTHI infection in the upper airway of a subject, wherein the presence of NTHi bacteria or NTHI infection is determined by the presence of at least one biomarker selected from OMP P2 and OMP P5 as determined by any of the preceding immunoassays.

[0046] In another aspect of the invention, the invention provides for immunoassays for detecting the presence of a nontypeable NTHI bacteria in the upper respiratory tract of a subject comprising the steps of a) obtaining a sample of secretions from the upper respiratory tract of the subject using a device comprising antibodies specific for at least one biomarker associated with the presence of a NTHI bacteria in the upper respiratory tract of the subject, wherein at least one biomarker is OMP P2 or OMP P5; b) detecting the presence of at least one biomarker associated with the presence of a NTHI bacteria in the upper respiratory tract of the subject to generate a protein profile; c) comparing the protein profile with a reference protein profile, wherein the reference protein profile identifies NTHI bacteria; and d)

determining whether the protein profile of the sample associates to the reference protein profile, wherein association is indicative of the presence of the NTHI bacteria in the upper respiratory tract of the subject.

[0047] The invention also provides for immunoassays for detecting the presence NTHI bacteria in the upper respiratory tract of a subject as described above further comprising a step of diagnosing the subject with a NTHI infection wherein the presence of NTHI bacteria in the upper respiratory tract of the subject is indicative of a NTHI infection.

[0048] The invention also provides for any of the preceding immunoassays, which further comprise the step of administering a therapeutic compound in an amount effective to treat the bacterial infection. Exemplary therapeutic compounds include antibiotics such as penicillin, erythromycin, amoxicillin, thimethoprim-sulfamethoxazole, doxycyline, cefpodoxime, cefuroxime, cefdinir, clarithromycin, azithromycin, levofloxacin, gatifloxacin, and moxifloxacin, alpha-adreneric agonists such as oxymetazoline hydrochloride, anticholinergic (parasympatholytic) agents such as ipratropium bromide, antihistamines such as chlorpheniramine maleate, beta-agonist bronchodilators, non-steroidal anti-inflammatory drugs, camphor, menthol, Echinacea, mast cell stabilizers such as cromolyn sodium, topical nasal steroids such as fluticasone propionate, budenoside, mometasone, triamcinolone, and dexamethasone, and zinc salts.

[0049] In another aspect of the invention, the invention provides for immunoassays for diagnosing a NTHI infection in the upper respiratory tract of a subject comprising the steps of a) obtaining a sample of secretions from the upper respiratory tract of the subject using a device comprising antibodies specific for at least one biomarker associated with the presence of a NTHI in the upper respiratory tract of the subject, wherein the at least one biomarker is OMP P2 or OMP P5; b) detecting the presence of at least one biomarker associated with the presence of a NTHI in the upper respiratory tract of the subject to generate a protein profile; c) comparing the protein profile with a reference protein profile, wherein the reference protein profile identifies NTHI; and d) determining whether the protein profile of the sample associates to the reference protein profile, wherein association is indicative of a NTHI infection in the upper respiratory tract of the subject.

[0050] The invention also provides for any of the preceding immunoassays further comprising the step of informing the subject of the presence of a NTHI bacteria or a NTHI infection in the upper respiratory tract of the subject. Exemplary therapeutic compounds include antibiotics such as penicillin, erythromycin, amoxicillin, thimethoprim-

sulfamethoxazole, doxycyline, cefpodoxime, cefuroxime, cefdinir, clarithromycin, azithromycin, levofloxacin, gatifloxacin, and moxifloxacin, alpha-adreneric agonists such as oxymetazoline hydrochloride, anticholinergic (parasympatholytic) agents such as ipratropium bromide, antihistamines such as chlorpheniramine maleate, beta-agonist bronchodilators, non-steroidal anti-inflammatory drugs, camphor, menthol, Echinacea, mast cell stabilizers such as cromolyn sodium, topical nasal steroids such as fluticasone propionate, budenoside, mometasone, triamcinolone, and dexamethasone, and zinc salts.

[0051] In addition, the sample used in any of the preceding immunoassays may be obtained using a sterile swab, sterile gauze, suction tube or a balloon catheter.

[0052] In another aspect of the invention, the invention provides for a device for obtaining a sample of secretions from the upper respiratory tract of a subject comprising a substrate presenting antibodies specific for at least one biomarker associated with the presence of a pathogenic bacteria in the upper respiratory tract of the subject.

[0053] The invention also provides for devices for carrying out any of the preceding methods of the invention or any of the preceding immunoassays of the invention which is used for obtaining a sample of secretions from the upper respiratory tract of a subject comprising a substrate presenting antibodies specific for biomarkers associated with the presence of a pathogenic bacteria in the upper respiratory tract of the subject.

[0054] In any of the preceding devices, the antibodies may be specific for OMP P2 or OMP P5, such as monoclonal antibodies that specifically bind NTHI OMP P2 or monoclonal antibodies that specifically bind NTHI OMP P5.

[0055] In another aspect of the invention, the invention provides for kits for carrying out any of the preceding methods or immunoassys. In one embodiment, the kits comprise a substrate presenting antibodies specific for at least one biomarker associated with the presence of a pathogenic bacteria or a bacterial infection in the upper respiratory tract of the subject. In another embodiment, the kits comprise devices for obtaining the sample from the sterile compartments within the upper respiratory tract of the subject and generating a protein profile associated with a pathogenic bacteria or bacterial infection in the upper respiratory tract of the subject. The kits may also comprise antibodies that specifically bind the protein biomarkers of interest and components for immunoassays to detect the protein biomarkers using these antibodies.

BRIEF DESCRIPTION OF DRAWINGS

[0056] Figure 1 depicts a silver stain of the distinct protein profile maintained over time in the NTHI biofilm supernatant.

[0057] Figure 2 depicts a Western blot analysis verifying the presence of the NTHI OMPs in NTHI biofilm supernatant.

[0058] Figure 3 depicts a Western blot analysis verifying the presence of OMP P2 and OMP P5 in the biofilm supernatant of various strains of NTHI.

DETAILED DESCRIPTION

[0059] The invention provides for methods with improved sensitivity and specificity for detecting and diagnosing bacterial sinusitis. In particular, the methods of invention comprise antibody-based bacterial detection of proteins within secretions of pathogenic biofilm located within the paranasal sinus cavities. These methods allow for the detection of trace quantities of signature proteins that identify specific bacterial pathogens from typically sterile sites in the paranasal sinus cavities. The methods of the invention provide for the ability to avoid broad-spectrum, empiric antibiotics which are often inappropriately given treat upper viral respiratory infections due to the difficulty in diagnosing bacterial sinusitis with a high sensitivity and high specificity. The methods of the invention are an improvement over typical bacterial cultures because these cultures have very low sensitivity for detecting bacterial biofilms and low specificity for distinguishing between commensal and pathogenic organisms.

[0060] The invention also provides for a device that involves delivering a wire through a balloon catheter to the typically sterile paranasal sinus cavities, sampling mucus from these sites, and rapidly detecting the presence of trace quantities of signature proteins that identify specific bacterial pathogens from these typically sterile sites. Upon obtaining the sample, an immunoassay may be run to generate a protein profile that is compared to a reference protein profile generated for the pathogenic bacteria known to cause chronic sinusitis or an infection of the upper respiratory tract.

Biomarkers

[0061] The term "biomarker" refers to a naturally occurring molecule, gene, or characteristic by which a particular pathological or physiological process, disease, or the like can be identified or characterized. The term "biomarker" may refer to a protein measured in

sample whose concentration reflects the severity or presence of some disease state. Biomarkers may be measured to identify risk for, diagnosis of or progression of a pathological or physiological process, disease or the like. Exemplary biomarkers include proteins, hormones, prohormones, lipids, carbohydrates, DNA, RNA and combinations thereof.

[0062] For example, biomarkers for NTHI pathogenic bacteria include outer membrane protein P2 (OMP P2: SEQ ID NO: 1), high molecular weight adhesin 1 (HMW1A; SEQ ID NO: 2), putative periplasmic chelated iron binding proteins (SEQ ID NO: 3), IgA-specific serine endopeptidase (SEQ ID NO: 4), outer membrane protein P5 (OMP P5; SEQ ID NO: 5), galactose-1-phosphate uridylyltransferase (SEQ ID NO: 6), HMWA (SEQ ID NO: 7), phosphate ABC transporter phosphate-binding protein (SEQ ID NO: 8), putative adhesin B precursor FimA (SEQ ID NO: 9), high molecular weight adhesin 2 (HMW2A; SEQ ID NO: 10), outer membrane protein P5 precursor (SEQ ID NO: 11) and outer membrane protein P1 (OMP P1; SEQ ID NO: 12).

[0063] The methods of the invention include detecting at least one biomarker, at least two biomarkers, at least three biomarkers, at least four biomarkers, at least five biomarkers or six or more biomarkers of the protein profile of a pathogenic bacteria. Detection of the protein biomarkers includes detecting full length or fragments of the protein biomarkers, including immunogenic or biologically active fragments. In particular, the methods of the invention include detecting at least OMP P2 and OMP 5 to generate a protein profile of NTHI bacteria.

[0064] The invention also provides biologically active or immunologically active variants of the amino acid sequences of the present invention; and "substantial equivalents" thereof (e.g., with at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, 86%, 87%, 88%, 89%, at least about 90%, 91%, 92%, 93%, 94%, typically at least about 95%, 96%, 97%, more typically at least about 98%, or most typically at least about 99% amino acid identity) that retain biological and/or immunogenic activity. Polypeptides encoded by allelic variants may have a similar, increased, or decreased activity compared to polypeptides encoded by the native polynucleotides.

[0065] The present invention further provides isolated polypeptides or peptides encoded by the nucleic acid fragments or by degenerate variants of the nucleic acid fragments. The term "degenerate variant" refers to nucleotide fragments which differ from a native nucleic acid fragment (e.g., an ORF) by nucleotide sequence but, due to the degeneracy of the genetic

code, encode an identical polypeptide sequence. Preferred nucleic acid fragments are the ORFs that encode proteins.

[0066] The invention also provides for polypeptides with one or more conservative amino acid substitutions that do not affect the biological and/or immunogenic activity of the polypeptide. Alternatively, the polypeptides are contemplated to have conservative amino acids substitutions which may or may not alter biological activity. The term "conservative amino acid substitution" refers to a substitution of a native amino acid residue with a nonnative residue, including naturally occurring and nonnaturally occurring amino acids, such that there is little or no effect on the polarity or charge of the amino acid residue at that position. For example, a conservative substitution results from the replacement of a non-polar residue in a polypeptide with any other non-polar residue. Further, any native residue in the polypeptide may also be substituted with alanine, according to the methods of "alanine scanning mutagenesis". Naturally occurring amino acids are characterized based on their side chains as follows: basic: arginine, lysine, histidine; acidic: glutamic acid, aspartic acid; uncharged polar: glutamine, asparagine, serine, threonine, tyrosine; and non-polar: phenylalanine, tryptophan, cysteine, glycine, alanine, valine, proline, methionine, leucine, norleucine, isoleucine. General rules for amino acid substitutions are set forth in Table 1 below.

Table 1
Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asn
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe,	Leu
Leu	Norleucine, Ile, Val, Met,	Leu
Lys	Arg, 1,4 Diaminobutyric	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Arg
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala,	Leu

[0067] The polypeptides may be encoded by nucleotide sequences that are substantially equivalent to the polynucleotides encoding the polypeptide biomarkers. Polynucleotides according to the invention can have, e.g., at least 65%, at least 70%, at least 75%, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically at least 90%, 91%, 92%, 93%, or 94% and even more typically at least 95%, 96%, 97%, 98% or 99% sequence identity to the native polynucleotide sequences.

[0068] Included within the scope of the nucleic acid sequences of the invention are nucleic acid sequence fragments that hybridize under stringent conditions to the nucleotide sequences encoding the polypeptide biomarkers or compliments thereof, which fragment is greater than about 5 nucleotides, preferably 7 nucleotides, more preferably greater than 9 nucleotides and most preferably greater than 17 nucleotides. Fragments of, e.g., 15, 17, or 20 nucleotides or

more that are selective for (i.e., specifically hybridize to any one of the polynucleotides of the invention) are contemplated. Probes capable of specifically hybridizing to a polynucleotide can differentiate polynucleotide sequences of the invention from other polynucleotide sequences in the same family of genes or can differentiate genes from other bacterial genes, and are preferably based on unique nucleotide sequences.

[0069] The term "stringent" is used to refer to conditions that are commonly understood in the art as stringent. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of stringent conditions for hybridization and washing are 0.015 M sodium chloride, 0.0015M sodium citrate at 65-68°C or 0.015 M sodium chloride, 0.0015M sodium citrate, and 50% formamide at 42 °C See Sambrook et al., Molecular Cloning: A Laboratory Manual, 2.sup.nd Ed., Cold Spring Harbor Laboratory, (Cold Spring Harbor, N.Y. 1989). More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used, however, the rate of hybridization will be affected. In instances wherein hybridization of deoxyoligonucleotides is concerned, additional exemplary stringent hybridization conditions include washing in 6x.SSC 0.05% sodium pyrophosphate at 37 °C (for 14-base oligos), 48 °C (for 17-base oligos), 55 °C (for 20-base oligos), and 60 °C. (for 23-base oligos).

[0070] Other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate, NaDodSO.sub.4, (SDS), ficoll, Denhardt's solution, sonicated salmon sperm DNA (or other non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these additives can be changed without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are usually carried out at pH 6.8-7.4, however, at typical ionic strength conditions, the rate of hybridization is nearly independent of pH. See Anderson et al., Nucleic Acid Hybridisation: A Practical Approach, Ch. 4, IRL Press Limited (Oxford, England). Hybridization conditions can be adjusted by one skilled in the art in order to accommodate these variables and allow DNAs of different sequence relatedness to form hybrids.

[0071] The sequences falling within the scope of the present invention are not limited to these specific sequences, but also include allelic and species variations thereof. Preferred computer program methods to determine identity and similarity between two sequences

include, but are not limited to, the GCG program package, including GAP (Devereux et al., Nucl. Acid. Res., 12:.387,-1984; Genetics Computer Group, University of Wisconsin, Madison, Wis.), BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol., 215: 403-410, 1990). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB/NILM/NIH Bethesda, MD 20894; Altschul et al., supra). The well known Smith Waterman algorithm may also be used to determine identity.

Methods of Generating Protein Profiles

[0072] The methods of the invention involve generating a protein profile of secretion samples obtained from the upper respiratory tract of a subject and generating protein profiles of pathogenic bacteria biofilm supernatants. The known pathogenic bacteria biofilm protein profiles may be used as reference protein profiles for use in the methods of the invention.

[0073] Separation of protein of interest from the other members of the protein profile may be accomplished by any number of techniques, such as sucrose gradient centrifugation, aqueous or organic partitioning (e.g., two-phase partitioning), non-denaturing gel electrophoresis, isoelectric focusing gel electrophoresis, capillary electrophoresis, isotachyphoresis, mass spectroscopy, chromatography (e.g., HPLC), polyacrylamide gel electrophoresis (PAGE, such as SDS-PAGE), gel permeation, ion-exchange spin columns, and the like. In these embodiments, SELDI, or other rapid analysis techniques, may be used for monitoring the purification process. Following purification, all potential biomarkers may be characterized by SDS PAGE and mass spectrometry and identified by peptide mapping and/or amino acid sequence analysis.

[0074] For example, the protein biomarkers may be separated by size or buoyant density gradient separation method, such as a discontinuous sucrose gradient, that separates the component polypeptides of the sample by the sizes of the complexes in which they participate. Sucrose gradients for the separation of proteins are well known, and may be modified as needed. Such modifications may include the use of a continuous, rather than discontinuous gradient, and different gradient conditions (for instance, different sucrose concentrations or different buffers). The length of the gradient can also be varied, with longer gradients expected to give better overall separation of proteins and protein complexes, and to provide a larger number of fractions that are then each individually analyzed using a denaturing system.

[0075] The individual protein biomarkers may be separated by electrophoresis based upon size (e.g., by SDS-PAGE or sizing gel). Other separation techniques may include aqueous two-phase partitioning and non-denaturing agarose gel electrophoresis separation. In other embodiments, separation employs denaturing system such as an isoelectric focusing (IEF) gel, capillary electrophoresis, or isotachyphoresis. Alternatively or additionally, two-dimensional electrophoretic analysis may be used (e.g., Wilkins et al., Proteome Research: New Frontiers in Functional Genomics, Springer-Verlag, Berlin, 1997). Proteins can be visualized on such gels using any of various stains known in the art (e.g., Trypan Blue or SyproRuby dye). Traditional buffering systems can also be used for separating proteins in the component fractionations of the described systems. The temperature, voltage, and amperage at which individual gels are run also can be modified, as can the speed and duration of gradient equilibration and centrifugation..

[0076] Purification of protein biomarkers be performed using traditional chromatographic techniques. In an embodiment, high pressure liquid chromatography (HPLC) may be used. Also, a combination of high pressure liquid chromatography (HPLC) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) may be used to purify the protein. The fractions may then be assayed for the protein of interest using SELDI or other methods.

[0077] A variety of methods may be used to generate the protein profile such as certain Matrix Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry technology, Surface Enhanced Laser Desorption/Ionization (SELDI) and Protein Chip Mass Spectrometry.

[0078] The methods may include steps for analyzing the protein profile. In an embodiment, analysis of the protein profile comprises a statistical analysis and other data manipulation techniques (e.g., signal processing, removal of noise). In some embodiments, techniques for analysis comprise computer statistical and data processing software. For example, analysis of the protein profile may comprise a determination of at least one of the molecular weight (mass), net charge, and or amount of the proteins in the sample.

[0079] The method may also comprise the step of comparing the protein profile for the subject's sample to a reference protein profile. In addition to biofilm protein profiles generated for known strains of bacteria, the reference profile may be from a healthy control subject who does not exhibit symptoms of the disease of interest (i.e., a negative control). The reference profile may be from a subject who has a disease of interest (i.e., a positive control). Also, the sample protein profile may be compared to a reference protein profile

isolated from the same subject, but at a different point in time (e.g., to monitor progression or remission of the disease). In yet other embodiments, the sample protein profile may be compared to a plurality of a reference protein profiles, as for example, reference profiles generated as diagnostic of a particular disease or disease subtype. In this way, it may be possible to determine whether the sample protein profile matches a particular protein or proteins of interest that are typical of any one disease or disease subtype.

Kits and Devices for Carrying Out the Methods of the Invention

[0080] The invention provides for kits for carrying out the methods and immunoassays of the invention. In one embodiment, the kits comprise devices for obtaining the secretion sample from the sterile compartments within the upper respiratory tract of the subject. The kits may also comprise antibodies that specifically bind the protein biomarkers of interest and components for immunoassays to detect the protein biomarkers using these antibodies. In addition, the kits may comprise substrates presenting antibodies specific for the protein biomarkers of interest. Furthermore, the kits may comprise instructions for carrying out the any of the methods or immunoassays of the invention.

[0081] In one embodiment, secretions from the upper respiratory tract may be obtained using sterile swabs or gauze. In another embodiment of the invention, the secretion sample may be collected using nasal washing methods. Alternatively, the secretion sample may be collected using a suction tube attached to an electric pump and a catheter inserted into the nasopharynx of the subject.

[0082] In another embodiment, the device for obtaining the secretion sample is a modified balloon catheter Seldinger technique that allows for collection of secretions from the sterile compartments within the upper respiratory tract of the subject. The balloon catheter may have a substrate presenting antibodies specific for the protein biomarkers of interest threaded into the catheter. In a further embodiment, a modified distal chip brochoesophagoscope or transnasal esophagoscope may be used in which a substrate presenting antibodies specific for the protein biomarkers of interest is threaded into the suction port of the device.

[0083] The invention provides for an immunoassay for detecting at least one biomarker that is specific for a biofilm protein profile for a pathogenic bacteria. For example, antibodies specific for two or more biomarkers within the protein profile are presented or absorbed to a solid substrate, and the secretion sample obtained from the upper airway of the

respiratory tract of a subject are contacted with the solid substrate and binding of the antibody to the substrate is detected.

[0084] Any type of immunoassay system known in the art may be used to detect the biomarkers of the protein profiles. Exemplary methods include, but not limited to: radioimmunoassays, ELISA assays, sandwich assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, fluorescent immunoassays, protein A immunoassays and immunoelectrophoresis assays and any other methods of generating a protein profile described herein. The immunoassays may be a sandwich assay in which the target analyte (biomarker of interest) is "sandwiched" between a labeled antibody and an antibody immobilized on the solid substrate. The immunoassay is read by observing the presence and amount of antigen-labeled antibody complex bound to the immobilized antibody. Another immunoassay may also be a "competition" type immunoassay, wherein an antibody immobilized on a solid surface is contacted with a sample (e.g., secretions from the upper respiratory tract) containing both an unknown quantity of antigen analyte (biomarker of interest) and with labeled antigen of the same type. The amount of labeled antigen bound on the solid substrate is then determined to provide an indirect measure of the amount of antigen analyte (biomarker of interest) in the sample. Such immunoassays are readily performed in a "dipstick" or other test device format (e.g., a flow-through or migratory dipstick or other test device design) for convenient use. For example, numerous types of dipstick immunoassays assays are described in U.S. Pat. No. 5,656,448.

[0085] The immune assays may be carried out on sheets, e.g. strips or sheet s of nitrocellulose or polyvinylidene difloride (PVDH) or other membranes, dipstick, wells *e.g.* 96-well plastic plates, or in tubes.

[0086] A device used in the methods and immunoassays of the invention can, for example, provide a color indication when the biomarker of interest is within the secretion sample from the upper respiratory tract of a subject. The device could be used in a clinical setting to quickly determine if a subject has a pathological bacteria or a bacterial infection in the upper respiratory tract. Alternatively, the methods and immunoassays of the present invention may be used in combination with a densitometer or generally a device for measuring light intensity, transmittance, reflection or refraction, or for measuring the wavelength of light as a measure of assay result. The densitometer or other device can provide rapid measurement of the optical density of the substrate within the device that have been contacted with the

secretions sample. In one embodiment, a change in color, density, or other parameter can be read by the naked eye.

[0087] The invention also may be carried out using a lateral-flow immunoassay which contains a device within the assay to extract the sample for analysis, and antibodies specific for the proteins within the protein profile of a pathogenic bacteria of interest. The invention also provides for a immunoassay device, for example, such as those described in US Patent Nos. 5,415,994 and 5,763,262, which comprise a protein profile identified for a particular pathogenic bacteria using any of the method of the invention. In particular, the invention provides for colorimetric immunoassays that allow for visual detection of the biomarkers of interest within the secretion sample. Visual detection allows for a rapid result which can be incorporated into a treatment plan for the infection.

[0088] A reference or standard protein profile may be used in the methods of the invention to compare the sample protein profile generated by the methods, immunoassays or kits of the invention. The reference or standard protein profile provides the concentration of a biomarker known to be present in the biofilm secretion of a pathogenic bacteria within the upper respiratory tract during an infection. A "calibrator" refers to immunoassays that detect known amounts of biomarkers of interest to generate a calibration curve to quantify the concentration of the biomarker in an unknown biological fluid.

[0089] The term "standard" or “reference” refers to immunoassays that measure biomarkers of interest from biological fluids known to be collected from a subject having a bacterial infection of the upper respiratory tract in a suitable quantitative form to control the quality of reagents contained in an immunoassay kit of the present invention. Other aspects and advantages of the present invention will be understood upon consideration of the following illustrative examples.

EXAMPLES

Example 1
Determination of Signature Protein Profile for Pathogenic Bacteria

[0090] Supernatants from Nontypeable *H. influenzae* (NTHI) biofilm were analyzed to determine the NTHI signature protein profile. NTHI strain 86-028NP was cultured in eight-well chamber slides for 10 days and the resulting supernatants were collected at 24 hours intervals. The proteins in the supernatants collected from NTHI biofilm cultures were

separated by SDS-PAGE and silver stain revealed a distinct protein profile maintained over time as shown in Figure 1.

[0091] The proteins isolated from NTHI biofilm supernatants were analyzed by nano-liquid chromatography/tandem mass spectrometry (LC-MS/MS). The molecular weights of the identified proteins were compared to the molecular weights of the known protein profile for the NTHI strain 86-028NP ((Bakaletz *et al. Infection and Immunity*, 56(2): 331-335, 1988), and the identified proteins were scored based on their association to the 86-028NP protein profile using Mascot (Matrix Science, Boston MA) according to the manufacturer's instructions. The results of this comparison are set out in Table 2 below. Several NTHI outer membrane proteins (OMPs) were identified (in bold), with predominance of major OMPs (bold italics): high molecular weight adhesins 1 and 2 (HMW1/HMW2), OMP P5, OMP P2, OMP P1, and IgA-protease.

[0092] In order to verify the presence of the NTHI OMPs in NTHI biofilm supernatants, Western blot analysis was carried out with antiserum against total OMPs, OMP P5 and OMP P2 (chinchilla polyclonal antibodies), as well as HMW1 and HMW2 proteins (monoclonal antibodies). This analysis verified the presence of multiple NTHI- specific OMPs in biofilm supernatants (see Figure 2).

Table 2:

IDENTIFIED PROTEIN	Score	Mass (kDa)	Accession #	SEQ ID NO:
Outer membrane protein P2	1227	39.9	*gi|68248747*	1
HMW1A, high molecular weight adhesin 1	1205	154.5	*gi|68250281*	2
putative periplasmic chelated iron binding protein	1089	32.4	*gi|301169065*	3
IgA-specific serine endopeptidase	948	197.5	*gi|68249575*	4
Outer membrane protein P5	886	38.4	*gi|68249712*	5
galactose-1-phosphate uridylyltransferase	791	34.0	*gi|145640927*	6
HMWA	720	160.5	*gi|68249817*	7
phosphate ABC transporter phosphate-binding protein	703	36.6	*gi|16273649*	8
putative adhesin B precursor FimA	402	35.0	*gi|3003012*	9
HMW2A, high molecular weight adhesin 2	326	160.7	*gi|68249817*	10
HMWA	321	160.5	*gi|5929966*	11
***Outer membrane protein P5*;** Precursor	283	37.7	*gi|585614*	12
Outer membrane protein P1	215	49.7	*gi|9716607*	13

[0093] One example of a signature protein profile of pathogenic NTHI biofilm is OMP P5, OMP P2, HMW1 and HMW2. Therefore, detection of these protein biomarkers in a secretion sample obtained from the upper respiratory tract is indicative of NTHI infection. Precise diagnosis of pathogenic bacterial infection, such as NTHI infection, in patients with upper airway infection will facilitate the selection of appropriate therapy and promote judicious prescription of antibiotics in order to achieve an early recovery in patients and to reduce the emergence of antibiotic-resistant infections in the community.

Example 2
Detection of NTHI Biofilm-Specific Proteins in Paranasal Sinus Infection

[0094] In order to determine the protein profile of a human patient suffering from sinusitis, secretion samples are obtained from the upper respiratory tract of the patients. These samples are analyzed as described in Example 1 for the presence of OMP P5, OMP P2, HMW1 and HMW2. The protein profile of the patients is compared with the reference protein profile generated from the supernatants of *in vitro* NTHI biofilms as described above.

Example 3
Identification of Protein Biomarkers Associated with other Bacteria Species

[0095] The methods described in Example 1 are carried out with the supernatants from biofilms of other pathogenic bacteria species such as *Streptococcus pneumonia*, *Moraxella catarrhalis*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*.

Example 4
Further Analysis of Determination of Signature Protein Profile for Pathogenic Bacteria

[0096] Supernatants from biofilm obtained from multiple stains of Nontypeable *H. influenzae* (NTHI) were analyzed to define the NTHI signature protein profile. NTHI strains 86-028NP, 1128MEE, 1714, 1748, 1885MEE and 2019 were cultured in eight-well chamber slides for 10 days and the resulting supernatants were collected at 24 hours intervals. The proteins in the supernatants collected from NTHI biofilm cultures were separated by SDS-PAGE and silver staining revealed a distinct protein profile maintained over time. Figure 3 depicts a Western blot using chinchilla anti-OMP P2 or anti-OMP P5 antibodies, which demonstrates that OMP P2 and OMP P5 are present in high levels in the biofilms of all NTHI strains tested.

[0097] The proteins isolated from NTHI biofilm supernatants were analyzed by nano-liquid chromatography/tandem mass spectrometry (LC-MS/MS). The molecular weights of

the identified proteins were compared to the molecular weights of the known protein profile for the NTHI strain 86-028NP ((Bakaletz *et al. Infection and Immunity*, 56(2): 331-335, 1988), and the identified proteins were scored based on their association to the 86-028NP protein profile using Mascot (Matrix Science, Boston MA) according to the manufacturer's instructions. The results of this comparison are set out in Table 3 below. These studies demonstrate that a preferred NTHI biofilm protein profile comprises OMP P2 and OMP P5.

Table 3:

P2 Fragment

Score	Description	Organism
2927	**Outer membrane protein P2**	*H. influenzae*
771	**Outer membrane protein P5**	*H. influenzae*
688	**Spermidine/putrescine-binding periplasmic protein 1**	*H. influenzae*
352	Keratin, type II cytoskeletal 2 epidermal	*Homo sapiens*
335	Trypsin	*Sus scrofa*
292	Protein mrp homolog	*H. influenzae*
165	3-dehydroquinate synthase	*H. influenzae*
143	Phenylalanyl-tRNA synthetase alpha chain	*H. influenzae*
105	Glutamate 5-kinase	*H. influenzae*
100	Aspartate-semialdehyde dehydrogenase	*H. influenzae*

P5 Fragment

Score	Description	Organism
1512	**Lipoprotein E**	*H. influenzae*
1105	**Outer membrane protein P2**	*H. influenzae*
718	**Hybrid peroxiredoxin hyPrx5**	*H. influenzae*
361	**Outer membrane protein P5**	*H. influenzae*
354	Trypsin OS=Sus scrofa	*Sus scrofa*
255	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	*H. influenzae*
168	Putative glutamine amidotransferase HI_1037	*H. influenzae*
167	Phosphate import ATP-binding protein PstB	*H. influenzae*
124	Dihydrodipicolinate reductase	*H. influenzae*
118	Ig kappa chain C region	*M. musculus*

[0098] The isolated proteins were also purified using cationic and gel chromatography . The purified OMP P2 and OMP P5 protein will be used to generate monoclonal antibodies for use in the methods, immunoassays and devices of the invention. It is critical that the antibodies used in the methods, immunoassays and devices of the invention be highly

specific. The currently available chinchilla polyclonal antibodies do not exhibit the specificity necessary for carrying out the methods of the invention.

Example 5
Generation of Monoclonal Antibodies

[0099] The purified OMP P2 and OMP P5 proteins described in Example 4 are used to generate monoclonal antibodies for use in the methods, immunoassays and devices of the invention using standard techniques well known in the art.

[00100] For example, a mouse is immunized intraperitoneally with the purified OMP P2 protein or purified OMP P5 protein. Four days later, the mouse is sacrificed and spleen cells are fused with murine myeloma cells using methods standard in the art. For example, hybridoma technology is described in Kohler *et al., Nature* 256: 495, the human B-cell hybridoma technique is described in Kozbor et al., *Immunol. Today* 4, 72 (1983), the EBV-hybridoma technique to produce human monoclonal antibodies is described in Cole *et al. Monoclonal Antibodies in Cancer Therapy* (1985) Allen R. Bliss, Inc., pages 77-96, and methods of screening combinatorial antibody libraries is described in Huse *et al., Science* 246, 1275 (1989).

[00101] The fused cells are cloned in a 96-well plate for single colony selection. Seven to ten days after fusion, culture supernatants from each well with colonies are assayed for the presence of anti-OMP P2 or anti-OMP P5 antibodies. Two to four weeks after cloning, supernatants from single cell colonies are screen for the presence of anti-OMP P2 or anti-OMP P5 antibodies again. Wells with positive reactions are further expanded into larger wells and eventually expanded into flasks to harvest more supernatant for further testing.

[00102] Hybridoma cells from the positive clones are injected into pristine mice for production of ascites. The monoclonal antibodies are purified from the ascites, and the specificity of the purified monoclonal antibodies is tested using standard assays known in the art.

Example 6
Immunoassays of the Invention

[00103] Anti-OMP P2 and OPM P5 monoclonal antibodies, as described in Example 5, are used to in order to determine the protein profile of a human patient suffering from sinusitis. Secretion samples are obtained from the upper respiratory tract of the patients. These samples are analyzed as described in Example 1 for the presence of at least OMP P5, OMP

P2, HMW1 or HMW2. The protein profile of the patients is compared with the reference protein profile generated from the supernatants of *in vitro* NTHI biofilms as described above

[00104] The sensitivity and specificity parameters for the use of anti-OMP P2 and anti-OPM P5 monoclonal antibodies, as described in Example 5, are determined against a gold-standard real-time PCR assay using hpd as a primer for the detection of non-typeable *Haemophilus influenzae* that has been shown to be 100% specific and sensitive for the detection of NTHI strains 86-028NP, 1128MEE, 1714, 1748, 1885MEE and 2019 and several clinical isolates of *Moraxella catarrhalis*.

[00105] Numerous modifications and variations in the practice of the invention are expected to occur to those of skill in the art upon consideration of the presently preferred embodiments thereof. Consequently, the only limitation which should be placed upon the scope of the invention are those which appear in the appended claims.

What is Claimed:

1. A method of detecting the presence of a pathogenic bacteria in the upper respiratory tract of a subject comprising the steps of

a) obtaining a sample of secretions from the upper respiratory tract of the subject;

b) generating a protein profile of the sample;

c) comparing the protein profile with a reference protein profile, wherein the reference protein profile identifies a pathogenic bacteria; and

d) determining whether the protein profile of the sample associates to the reference protein profile, wherein association is indicative of the presence of the pathogenic bacteria in the upper respiratory tract of the subject.

2. The method of claim 1 further comprising the step of administering a therapeutic compound to reduce or eliminate the pathogenic bacteria in the upper respiratory tract of the subject.

3. The method of claim 1 or 2 further comprising the step of informing the subject of the presence or absence of the pathogenic bacteria in the upper respiratory tract.

4. The method of any one of claims 1-3 further comprising the step of diagnosing the subject with a bacterial infection, wherein the presence of the pathogenic bacteria in the upper respiratory tract of the subject is indicative of a bacterial infection.

5. A method of diagnosing a bacterial infection in the upper respiratory tract of a subject comprising the steps of

a) obtaining a sample of secretions from the upper respiratory tract of the subject;

b) generating a protein profile of the sample;

c) comparing the protein profile of the sample with a reference protein profile, wherein the reference protein profile indentifies a pathogenic bacteria; and

d) determining whether the protein profile of the sample associates to the reference protein profile; wherein association is indicative of a bacterial infection in the upper respiratory tract of the subject.

6. The method of claim 5 further comprising the step of informing the subject of the diagnosis of a bacterial infection in the upper respiratory tract.

7 The methods of claims 5 and 6 further comprising the step of administering a therapeutic compound in an amount effective to treat the bacterial infection.

8. The method of any one claims 1-7, wherein the pathogenic bacteria is *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus, Pseudomonas aeruginosa* or S*tenotrophomonas maltophilia.*

9. Use of a therapeutic compound for the preparation of a medicament to reduce or eliminate the pathogenic bacteria in the upper respiratory tract of a subject or to treat a bacterial infection in the upper respiratory tract of a subject, wherein the subject has a protein profile that associates to a reference protein profile, and wherein the association is indicative of the presence of the pathogenic bacteria or bacterial infection in the upper respiratory tract of the subject as determined by the method of any one of claims 1-8.

10. A therapeutic composition for the reduction or elimination of a pathogenic bacteria in the upper respiratory tract of a subject or for the treatment of a bacterial infection in the upper respiratory tract of a subject, wherein the subject has a protein profile that associates to a reference protein profile, and wherein the association is indicative of the presence of the pathogenic bacteria or bacterial infection in the upper respiratory tract of the subject tract of the subject as determined by the method of any one of claims 1-8.

11. A method of detecting the presence of nontypeable *Haemophilus influenzae* (NTHI) bacteria in the upper respiratory tract of a subject comprising the steps of

a) obtaining a sample of secretions from the upper respiratory tract of the subject; and

b) detecting the presence of at least one biomarker in the sample, wherein the biomarker is OMP P2 or OMP P5,

wherein the presence of at least one biomarker indicates the presence of NTHI bacteria in the upper respiratory tract of the subject.

12. The method of claim 11 further comprising the step of informing the subject of the presence or absence of NTHI bacteria in the upper respiratory tract of the subject.

13. The method of claim 11 or 12 further comprising the step of diagnosing the subject with a NTHI infection, wherein the presence of NTHI bacteria in the upper respiratory tract of the subject is indicative of a NTHI infection in the upper respiratory tract of the subject.

14. The method of any one of claims 11-13 further comprising the step of administering a therapeutic compound to reduce or eliminate the NTHI bacteria in the upper respiratory tract of the subject.

15. A method of diagnosing nontypeable *Haemophilus influenzae* (NTHI) infection in the upper respiratory tract of a subject comprising the steps of

a) obtaining a sample of secretions from the upper respiratory tract of the subject, and

b) detecting the presence of at least one biomarker in the sample, wherein the biomarkers are selected from OMP P2 and OMP P5,

wherein the presence of at least one biomarker indicates a NTHI infection in the upper respiratory tract of the subject.

16. The method of claim 15 further comprising the step of informing the subject of the diagnosis of a NTHI infection in the upper respiratory tract of the subject.

17. The methods of claims 15 or 16 further comprising the step of administering a therapeutic compound in an amount effective to treat the NTHI infection.

18. Use of a therapeutic compound for the preparation of a medicament to reduce or eliminate NTHI bacteria in the upper respiratory tract of a subject or to treat a NTHi infection in the upper respiratory tract of a subject, wherein the presence of NTHI bacteria or a NTHI infection is determined by the presence of at least one biomarker selected from OMP P2 and OMP P5 as determined by the method of any one of claims 11-17.

19. A therapeutic composition for the reduction or elimination of NTHI bacteria or for the treatment of NTHI infection in the upper respiratory tract of a subject, wherein the presence of NTHi bacteria or NTHI infection is determined by the presence of at least one biomarker selected from OMP P2 and OMP P5 as determined by the method of any one of claims 11-17.

20. The method, use or composition of any one of claims 1-19, wherein the sample is collected from the paranasal sinus cavity.

21. The method, use or composition of any of one of claims 1-20, wherein the subject is suffering from chronic sinusitis.

22. The method, use or composition of any one of claims 1-21, wherein the sample is collected using sterile swabs, sterile gauze, nasal washing, suction tube or a balloon catheter.

23. The method, use or composition of any one of claims 1-22, wherein the biomarker is detected using a monoclonal antibody.

24. The method, use or composition of any one of claims 1-23, wherein an immunoassay is used to detect the biomarker.

25. The method, use or composition of any one of claim 1-24, wherein the sample is collected with a device comprising a substrate presenting antibodies specific for the biomarkers.

26. The method, use or composition of claim 25, wherein the device is a balloon catheter and the substrate is threaded into the suction port of the catheter.

27. A immunoassay for detecting the presence of a pathogenic bacteria in the upper respiratory tract of a subject comprising the steps of

a) obtaining a sample of secretions from the upper respiratory tract of the subject using a device comprising antibodies specific for at least one biomarker associated with the presence of a pathogenic bacteria in the upper respiratory tract of the subject;

b) detecting the presence of at least one biomarker associated with the presence of a pathogenic bacteria in the upper respiratory tract of the subject to generate a protein profile;

c) comparing the protein profile with a reference protein profile, wherein the reference protein profile identifies a pathogenic bacteria; and

d) determining whether the protein profile of the sample associates to the reference protein profile, wherein association is indicative of the presence of the pathogenic bacteria in the upper respiratory tract of the subject.

28. The immunoassay of claim 27 further comprising a step of diagnosing the subject with a bacterial infection wherein the presence of the pathogenic bacteria in the upper respiratory tract of the subject is indicative of a bacterial infection.

29. A immunoassay for diagnosing a bacterial infection in the upper respiratory tract of a subject comprising the steps of

a) obtaining a sample of secretions from the upper respiratory tract of the subject using a device comprising antibodies specific for at least one biomarker associated with the presence of a pathogenic bacteria in the upper respiratory tract of the subject;

b) detecting the presence of at least one biomarker associated with the presence of a pathogenic bacteria in the upper respiratory tract of the subject to generate a protein profile;

c) comparing the protein profile with a reference protein profile, wherein the reference protein profile identifies a pathogenic bacteria; and

d) determining whether the protein profile of the sample associates to the reference protein profile, wherein association is indicative of a bacterial infection in the upper respiratory tract of the subject.

30. The immunoassay of any one of claims 27-29 further comprising the step of informing the subject of the presence of a pathogenic bacteria or bacterial infection in the upper respiratory tract of the subject.

31. The immunoassay of any one of claims 27-30 further comprising the step of administering a therapeutic compound in an amount effective to treat the bacterial infection.

32. The immunoassay of any one claims 27-31, wherein the pathogenic bacteria is *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus, Pseudomonas aeruginosa* or S*tenotrophomonas maltophilia.*

33. Use of a therapeutic compound for the preparation of a medicament to reduce or eliminate a pathogenic bacteria in the upper respiratory tract of a subject or treat a bacterial infection in the upper respiratory tract of a subject, wherein the subject has a protein profile that associates to a reference protein profile, and wherein the association is indicative of the presence of the pathogenic bacteria or bacterial infection in the upper respiratory tract of the subject tract as determined by the immunoassay of any one of claims 27-32.

34. A therapeutic composition for the reduction or elimination of a pathogenic bacteria in the upper respiratory tract of a subject or for the treatment of a bacterial infection in the upper respiratory tract of a subject, wherein the subject has a protein profile that associates to a reference protein profile, and wherein the association is indicative of the presence of the pathogenic bacteria or bacterial infection in the upper respiratory tract of the subject as determined by the immunoassay of any one of claims 27-32.

35. A immunoassay for detecting the presence of a nontypeable *Haemeophilus influenza* (NTHI) bacteria in the upper respiratory tract of a subject comprising the steps of

a) obtaining a sample of secretions from the upper respiratory tract of the subject using a device comprising antibodies specific for at least one biomarker associated with the presence of a NTHI bacteria in the upper respiratory tract of the subject, wherein at least one biomarker is OMP P2 or OMP P5;

b) detecting the presence of at least one biomarker associated with the presence of a NTHI bacteria in the upper respiratory tract of the subject to generate a protein profile;

c) comparing the protein profile with a reference protein profile, wherein the reference protein profile identifies NTHI bacteria; and

d) determining whether the protein profile of the sample associates to the reference protein profile, wherein association is indicative of the presence of the NTHI bacteria in the upper respiratory tract of the subject.

36. The immunoassay of claim 35 further comprising a step of diagnosing the subject with NTHI infection wherein the presence of NTHI bacteria in the upper respiratory tract of the subject is indicative of a NTHI infection.

37. The immunoassay of claim 35 or 36 further comprising the step of administering a therapeutic compound in an amount effective to treat the bacterial infection.

38. A immunoassay for diagnosing nontypable *Haemophilus influenae* (NTHI) infection in the upper respiratory tract of a subject comprising the steps of

a) obtaining a sample of secretions from the upper respiratory tract of the subject using a device comprising antibodies specific for at least one biomarker associated with the presence of a NTHI in the upper respiratory tract of the subject, wherein the at least one biomarker is OMP P2 or OMP P5;

b) detecting the presence of at least one biomarker associated with the presence of a NTHI in the upper respiratory tract of the subject to generate a protein profile;

c) comparing the protein profile with a reference protein profile, wherein the reference protein profile identifies NTHI; and

d) determining whether the protein profile of the sample associates to the reference protein profile, wherein association is indicative of a NTHI infection in the upper respiratory tract of the subject.

39. The immunoassay of any one of claims 35-38 further comprising the step of informing the subject of the presence of a NTHI bacteria or NTHI infection in the upper respiratory tract of the subject.

40. The immunoassay of any one of claims 35-39 further comprising the step of administering a therapeutic compound in an amount effective to treat the bacterial infection

41. The immunoassay of any one of claims 35-40, wherein the sample is obtained using a sterile swab, sterile gauze, suction tube or a balloon catheter.

42. Use of a therapeutic compound for the preparation of a medicament to reduce or eliminate NTHI baceteria in the upper respiratory tract of a subject or to treat a NTHi infection in the upper respiratory tract of a subject, wherein the presence of NTHi bacterial or a NTHi infection is determined by the presence of at least one biomarker selected from OMP P2 and OMP P5 as determined by the immunoassay of any one of claims 35-40.

43 A therapeutic composition for the reduction or elimination of NTHI bacteria in the upper respiratory tract of a subject or for the treatment of NTHI infection in the upper airway of a subject, wherein the presence of NTHi bacteria or NTHI infection is determined by the presence of at least one biomarker selected from OMP P2 and OMP P5 as determined by the immunoassay of any one of claims 29- 35.

44. A device for obtaining a sample of secretions from the upper respiratory tract of a subject comprising a substrate presenting antibodies specific for at least one biomarker

associated with the presence of a pathogenic bacteria in the upper respiratory tract of the subject.

45. A device for obtaining a sample of secretions from the upper respiratory tract of a subject comprising a substrate presenting antibodies specific for biomarkers associated with the presence of a pathogenic bacteria in the upper respiratory tract of the subject for carrying out any of the methods of claims 1-8, 11-17 and 20-26, or the immunoassays of claims 27-31 and 35-41.

46. The device of claims 44 or 45wherein the antibodies are specific for OMP P2 or OMP P5.

47. A kit for carrying out a method of any one of claims 1-8, 11-17 and 20-26, or the immunoassays of any one of claims 27-31 and 35-41comprising a substrate presenting antibodies specific for at least one biomarker associated with the presence of a pathogenic bacteria or a bacterial infection in the upper respiratory tract of the subject.

48. A kit for carrying of a method of any one of claims 1-8, 11-17 and 20-26, or the immunoassays of any one of claims 27-31 and 35-41comprising a device for obtaining a sample of secretions from the upper respiratory tract from a subject and generating a protein profile associated with a pathogenic bacteria or bacterial infection in the upper respiratory tract of the subject.

49. A kit for carrying out a method of any one of claims 1-8, 11-17 and 20-26, or the immunoassays of any one of claims 27-31 and 35-41 comprising a substrate presenting antibodies specific for generating a protein profile associated with a pathogenic bacteria or bacterial infection in the upper respiratory tract of the subject.

50. The kit of claim 49, wherein the device is a sterile swab, sterile gauze, suction tube or a balloon catheter.

51. The kit of any one of claims 47-50 wherein the pathogenic bacteria is *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus, Pseudomonas aeruginosa* or S*tenotrophomonas maltophilia*.

Figure 1

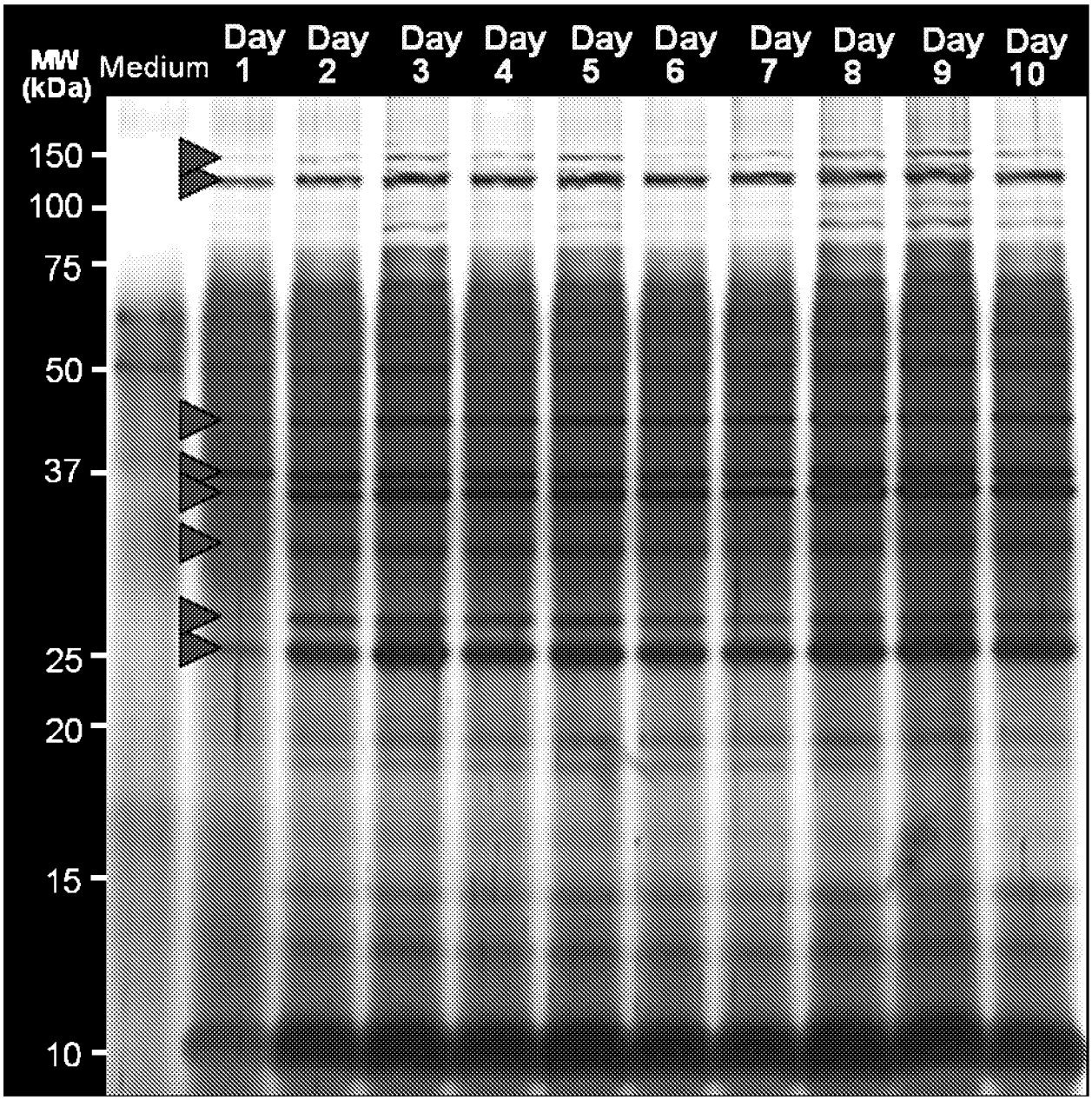
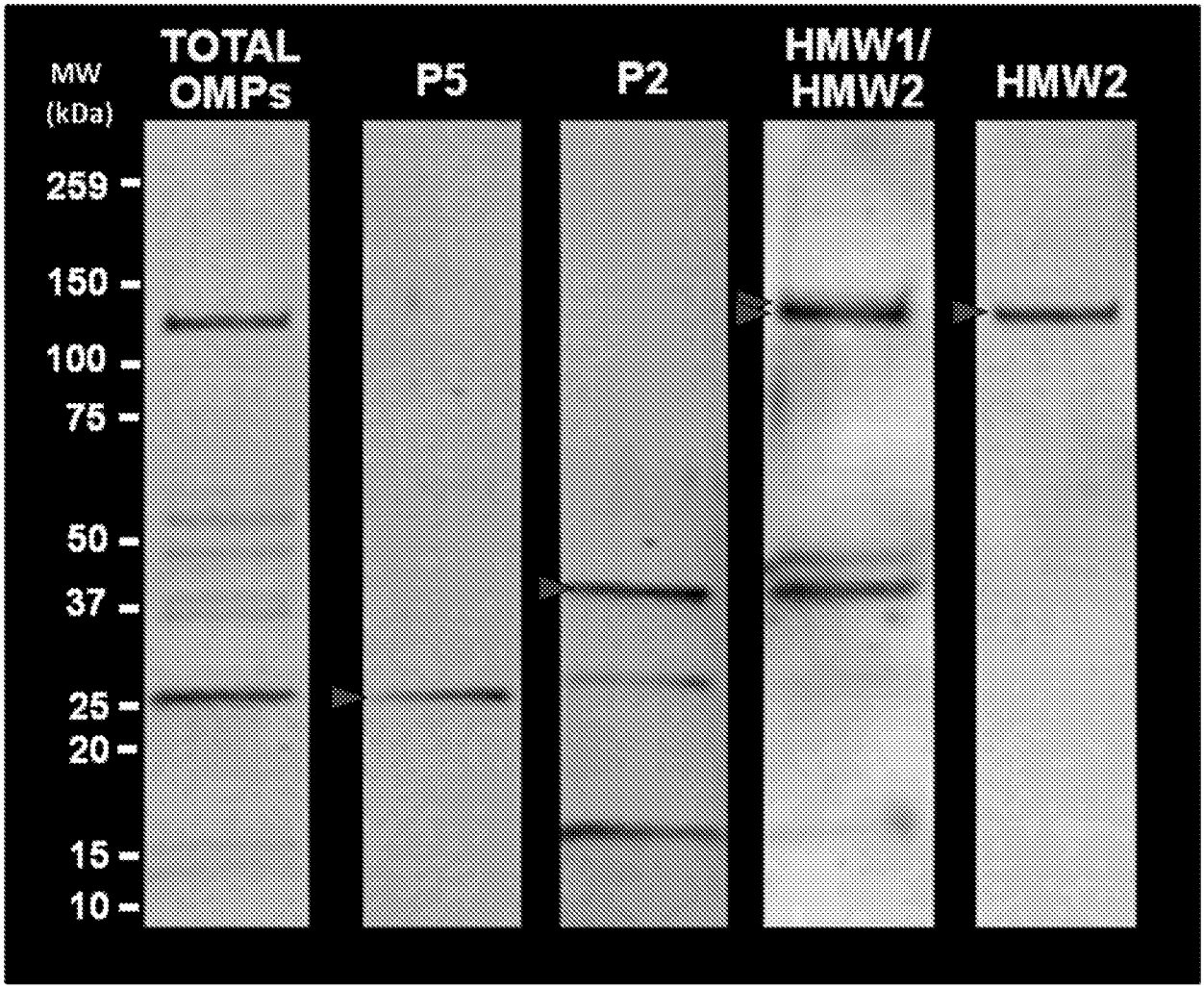

MW (kDa)
Medium
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
150
100
75
50
37
25
20
15
10

Figure 2


MW
(kDa)
TOTAL OMPs
P5
P2
HMW1/ HMW2
HMW2
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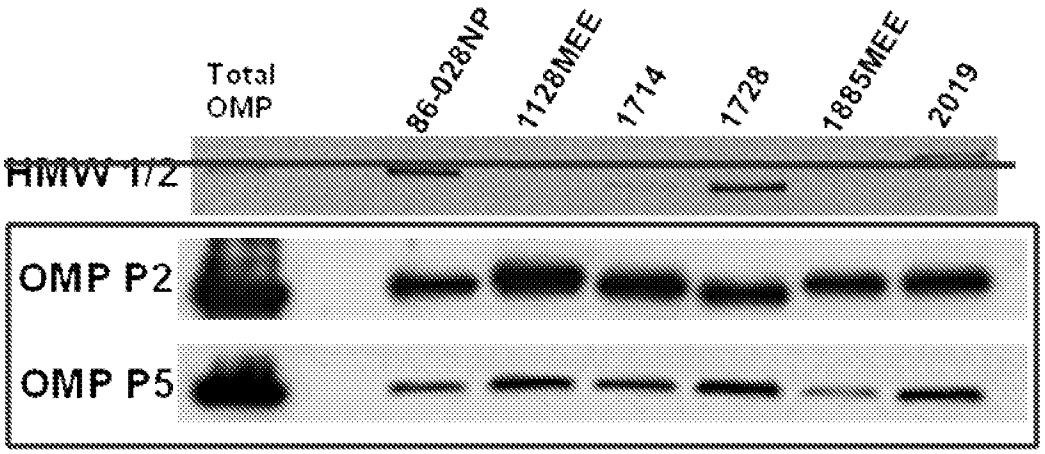
150
100
75
50
37
25
20
15
10

Figure 3


Total OMP
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1714
1728
1885MEE
2019
HMW 1/2
OMP P2
OMP P5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/040910

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68 G01N33/569
ADD.

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)
G01N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ALBERTO VILLASEÑOR-SIERRA ET AL: "Outer Membrane Protein Profiles of Paired Nasopharyngeal and Middle Ear Isolates of Nontypable Haemophilus influenzae from Mexican Children with Acute Otitis Media", CLINICAL INFECTIOUS DISEASES, vol. 28, no. 2, 1 February 1999 (1999-02-01), pages 267-273, XP055035558, ISSN: 1058-4838, DOI: 10.1086/515098	1-8, 20-26
Y	the entire document, particularly abstract, page 268, figures 1 to 4	11-17, 35-41

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[X] Further documents are listed in the continuation of Box C.

[X] 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
16 August 2012	22/10/2012

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KRASAN GP ET AL: "Adhesin Expression in Matched Nasopharyngeal and Middle Ear Isolates of Nontypeable Haemophilus influenzae from Chrildren with Acute Otitis Media", INFECTION AND IMMUNITY, vol. 67, no. 1, 1999, pages 449-454, XP002681883,	1-8, 20-32
Y	abstract; figure 1	11-17, 35-41

A	OLIVEIRA SIMONE ET AL: "Computer-based analysis of Haemophilus parasuis protein fingerprints", CANADIAN JOURNAL OF VETERINARY RESEARCH, CANADIAN VETERINARY MEDICAL ASSOCIATION, OTTAWA, CA , vol. 68, no. 1 1 January 2004 (2004-01-01), pages 71-75, XP008154833, ISSN: 0830-9000 Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1142133/pdf/cjvr68pg071.pdf the whole document	1-8, 11-17, 20-32, 35-41

A	QU JUN ET AL: "Proteomic expression profiling of Haemophilus influenzae grown in pooled human sputum from adults with chronic obstructive pulmonary disease reveal antioxidant and stress responses", BMC MICROBIOLOGY, BIOMED CENTRAL, LONDON, GB, vol. 10, no. 1, 1 June 2010 (2010-06-01), page 162, XP021073045, ISSN: 1471-2180, DOI: 10.1186/1471-2180-10-162 abstract; table 1	1-8, 11-17, 20-32, 35-41

A	GALLAHER TIMOTHY K ET AL: "Identification of biofilm proteins in non-typeable Haemophilus Influenzae", BMC MICROBIOLOGY, BIOMED CENTRAL, LONDON, GB, vol. 6, no. 1, 19 July 2006 (2006-07-19), page 65, XP021014897, ISSN: 1471-2180, DOI: 10.1186/1471-2180-6-65 the whole document	1-8, 11-17, 20-32, 35-41

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/040910

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2002/164354 A1 (BARENKAMP STEPHEN J [US]) 7 November 2002 (2002-11-07) paragraph [0036] - paragraph [0048]; figures 21-23 -----	1-8, 11-17, 20-32, 35-41

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/040910

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.:
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:

see additional sheet

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 an 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.:

11-17, 35-41(completely); 1-8, 20-32(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 11-17, 35-41(completely); 1-8, 20-32(partially)

   method of detecting the presence of a pathogenic bacteria and method of diagnosing a bacterial infection in the upper respiratory tract, wherein the bacteria is (nontypeable) Haemophilus influenzae

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2. claims: 1-8, 20-32(all partially)

   method of detecting the presence of a pathogenic bacteria and method of diagnosing a bacterial infection in the upper respiratory tract, wherein the bacteria is Streptococcus pneumoniae

   ---

3. claims: 1-8, 20-32(all partially)

   method of detecting the presence of a pathogenic bacteria and method of diagnosing a bacterial infection in the upper respiratory tract, wherein the bacteria is Moraxella catarrhalis

   ---

4. claims: 1-8, 20-32(all partially)

   method of detecting the presence of a pathogenic bacteria and method of diagnosing a bacterial infection in the upper respiratory tract, wherein the bacteria is Staphylococcus aureus

   ---

5. claims: 1-8, 20-32(all partially)

   method of detecting the presence of a pathogenic bacteria and method of diagnosing a bacterial infection in the upper respiratory tract, wherein the bacteria is Pseudomonas aeruginosa

   ---

6. claims: 1-8, 20-32(all partially)

   method of detecting the presence of a pathogenic bacteria and method of diagnosing a bacterial infection in the upper respiratory tract, wherein the bacteria is Stenotrophomanos maltophilia

   ---

7. claims: 1-7, 20-31(all partially)

   method of detecting the presence of a pathogenic bacteria and method of diagnosing a bacterial infection in the upper

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

respiratory tract, wherein the bacteria is any other pathogenic bacteria

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8. claims: 18, 19, 33, 34, 42, 43(completely); 9, 10, 20-26(partially)

Therapeutic composition for reduction or elimination of a pathogenic bacteria in the upper respiratory tract, wherein the bacteria is (nontypeable) Haemophilus influenzae

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9. claims: 9, 10(partially)

Therapeutic composition for reduction or elimination of a pathogenic bacteria in the upper respiratory tract wherein the bacteria is Streptococcus pneumoniae

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10. claims: 9, 10(partially)

Therapeutic composition for reduction or elimination of a pathogenic bacteria in the upper respiratory tract wherein the bacteria is Moraxella catarrhalis

---

11. claims: 9, 10(partially)

Therapeutic composition for reduction or elimination of a pathogenic bacteria in the upper respiratory tract, wherein the bacteria is Staphylococcus aureus

---

12. claims: 9, 10(partially)

Therapeutic composition for reduction or elimination of a pathogenic bacteria in the upper respiratory tract. wherein the bacteria is Pseudomonas aeruginosa

---

13. claims: 9, 10(partially)

Therapeutic composition for reduction or elimination of a pathogenic bacteria in the upper respiratory tract, wherein the bacteria is Stenotrophomonas maltophilia

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14. claims: 9, 10(partially)

Therapeutic composition for reduction or elimination of a pathogenic bacteria in the upper respiratory tract, wherein the bacteria is any other pathogenic bacteria

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15. claims: 44-46

device for obtaining a sample of secretions from the upper respiratory tract of a subject

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16. claims: 47-51

kit for carrying out the claimed methods

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/040910

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002164354 A1	07-11-2002	US 2002164354 A1	07-11-2002
		US 2005053618 A1	10-03-2005