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Emerging novel and antimicrobial-resistant respiratory tract infections: new drug development and therapeutic options

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The emergence and spread of antimicrobial-resistant bacterial, viral, and fungal pathogens for which diminishing treatment options are available is of major global concern. New viral respiratory tract infections with epidemic potential, such as severe acute respiratory syndrome, swine-origin influenza A H1N1, and Middle East respiratory syndrome coronavirus infection, require development of new antiviral agents. The substantial rise in the global numbers of patients with respiratory tract infections caused by pan-antibiotic-resistant Gram-positive and Gram-negative bacteria, multidrug-resistant *Mycobacterium tuberculosis*, and multiazole-resistant fungi has focused attention on investments into development of new drugs and treatment regimens. Successful treatment outcomes for patients with respiratory tract infections across all health-care settings will necessitate rapid, precise diagnosis and more effective and pathogen-specific therapies. This Series paper describes the development and use of new antimicrobial agents and immune-based and host-directed therapies for a range of conventional and emerging viral, bacterial, and fungal causes of respiratory tract infections.

Lancet Infect Dis 2014

Published Online
September 2, 2014
http://dx.doi.org/10.1016/S1473-3099(14)70828-X

See Online/Comment
http://dx.doi.org/10.1016/S1473-3099(14)70899-0

This is the fifth in a Series of five papers on emerging respiratory tract infections

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Introduction

The emergence of difficult-to-treat known and novel bacterial, viral, and fungal respiratory tract pathogens with epidemic potential is of major global concern. Treatment options are limited by increasing antimicrobial-drug resistance. However, new viral infections causing severe respiratory tract disease with pandemic potential have focused global attention.[1] A substantial rise in the number of patients with multidrug-resistant pulmonary tuberculosis[2] and pan-drug-resistant bacteria[3] has been noted. Increasing use of immunosuppressive agents, broad-spectrum antibiotics, and anticancer agents, coupled with resistance to azoles, has led to an increase in the number of invasive pulmonary fungal infections[4] with resultant high morbidity and mortality. Successful treatment outcomes for patients with respiratory tract infections across all health-care settings require appropriate, effective, and pathogen-specific drug or alternative treatments. We describe a range of conventional and emerging viral, bacterial, and fungal causes of respiratory tract infections for which new antimicrobial drugs and immune-based and host-directed therapies are being developed and studied.

Viral respiratory tract infections

The outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV),[5] re-emergence of avian influenza A H5N1,[6] global circulation of oseltamivir-resistant seasonal influenza A H1N1,[7] and subsequent emergence of the pandemic influenza A H1N1 strain pdm09 virus (which continues to circulate),[8] have shown the potential limitations of current antiviral treatments for severe respiratory viral infections. Epidemic waves of avian influenza A H7N9,[9] sporadic cases of avian influenza A H10N8,[10] the ongoing outbreak of Middle East respiratory syndrome coronavirus (MERS-CoV) infection, and the burden of common respiratory viruses[11]—such as seasonal influenza, respiratory syncytial virus, rhinoviruses, and adenoviruses—show that the development of more effective therapies to reduce morbidity and mortality is urgently needed. Research is focused on the repurposing of available antiviral drugs for generic or specific use and for

Key messages

- Respiratory tract infections are among the top two causes of morbidity and mortality worldwide. Antimicrobial-resistant species of bacteria, viruses, and fungi continue to emerge globally.
- A substantial rise in the numbers of cases of multidrug-resistant bacteria, azole-resistant fungi, and oseltamivir-resistant influenza A H1N1 causing respiratory tract infections has been identified, showing the potential limitations of current antibiotic, antiviral, and antifungal treatments for severe respiratory tract infections.
- Epidemic waves of avian influenza A H7N9 virus, sporadic cases of avian influenza A H10N8, and the ongoing outbreak of Middle East respiratory syndrome coronavirus infection show an urgent need for the development of more effective antivirals.
- Research is focused on repurposing available antiviral drugs for generic or specific use, or combination use with other adjunct interventions such as immunomodulators and host-directed therapies.
- Only one class of effective antiviral agents are approved for prevention and treatment of influenza in most countries: neuraminidase inhibitors (oseltamivir, peramivir, zanamivir, and laninamivir).
- Antibiotic treatment options are limited for pan-antibiotic resistant Gram-negative bacteria, and new antibacterial antibiotic pipeline remains thin.
- Increased investments into development of new antibacterial drugs and other antibacterial innovations and for more prudent use of existing antibiotics are required worldwide.
- Development of new therapeutic options needs to be coupled to international regulations on the use and prescription of antimicrobial drugs.

	Spectrum	Main mechanism of action	Antiviral resistance in clinical influenza isolates	Route of delivery	Pharmacokinetic features	Main adverse effects
Amantadine	Influenza A	Inhibition of M2 ion channel function, preventing virion uncoating	Widespread*	Oral	High oral bioavailability; long plasma elimination half-life (8–12 h); renal excretion of unchanged drug; dose adjustment required in renal dysfunction	CNS effects (including confusion, seizure, and psychosis), gastrointestinal effects, hypotension
Rimantadine	Influenza A	Inhibition of M2 ion channel function, preventing virion uncoating	Widespread*	Oral	High oral bioavailability; prolonged plasma elimination half-life (≥24 h); hepatic metabolism and renal excretion; dose adjustment required in severe hepatic and renal dysfunction	Gastrointestinal effects, CNS effects (lower risk than amantadine)
Oseltamivir	Influenza A and B	Inhibition of enzymatic action of viral neuraminidase†	Uncommon (1–2% in community isolates)‡	Oral	Rapid absorption of ethyl ester prodrug (phosphate) with conversion by gastrointestinal tract, hepatic, and blood esterases to the active carboxylate; peak concentrations at 3–4 h; renal excretion of both; carboxylate plasma elimination half-life of 8–10 h; dose adjustment required in renal dysfunction and young children	Gastrointestinal effects, insomnia, CNS effects (rare); anaphylaxis, severe skin reactions (rare)
Zanamivir	Influenza A and B	Inhibition of enzymatic action of viral neuraminidase*	Rare (<0·001% of community isolates)	Inhaled, nebulised, intravenous	Commercial inhaler delivers roughly 15% to lower respiratory tract; sputum concentrations detectable to 24 h; systemic bioavailability less than 20%; intravenous zanamivir excreted renally with plasma elimination half-life of roughly 2 h; dose adjustment required in renal insufficiency	Cough, bronchospasm, allergic reactions; lactose-containing commercial formulation should not be used in patients undergoing mechanical ventilation
Peramivir§	Influenza A and B	Inhibition of enzymatic action of viral neuraminidase*	Uncommon	Intravenous	Median peak and trough plasma concentrations of around 51 500 μg/mL and 46 μg/mL after 600 mg dose; predominantly renal excretion; dose adjustment required in renal insufficiency	Gastrointestinal and possible CNS effects; decreased polymorphonuclear counts
Laninamivir¶	Influenza A and B	Inhibition of enzymatic action of viral neuraminidase*	Rare	Inhaled	Octanoate prodrug converted to laninamivir in airway, prolonged detection in epithelial lining fluid; systemic bioavailability roughly 15%; plasma elimination half-life of around 3 days	Gastrointestinal effects, dizziness
Favipiravir/T-705‖	Influenza A, B, and C and many other RNA viruses	Undergoes intracellular ribosylation and phosphorylation to active triphosphate form and selectively inhibits RNA-dependent RNA polymerase of influenza virus; also induces lethal mutagenesis	Not reported	Oral	Good oral bioavailability; parent metabolised to inactive moiety by host aldehyde oxidase and also inhibitor of aldehyde oxidase (favipiravir's metabolic enzyme); loading dose necessary; more than 65% excreted by kidneys as metabolite by 48 h	Dose-related hyperuricaemia; restricted use in pregnancy
DAS181	Influenza A and B and parainfluenza viruses	Sialidase that destroys receptors for viral haemagglutinin; novel fusion construct that includes the catalytic domain from *Actinomyces viscosus* sialidase linked with an epithelium-anchoring domain of human amphiregulin; this sialidase removes both α-2,6-linked and α-2,3-linked sialic acids from cellular receptors	Not reported	Inhaled	In ex-vivo human airway epithelium and human bronchial tissue, the inhibitory effect of DAS181 treatment lasts for 2 days or more; tracheobronchial delivery and degree of systemic absorption depend on particle size	Increased alkaline phosphatase because of reduced clearance; no associated increases in transaminases
Nitazoxanide	Influenza A and B and other RNA viruses	Inhibition of haemagglutinin maturation; immunomodulation and perhaps other antiviral actions.	Not reported	Oral	Plasma esterases metabolise it into active desacetyl derivative tizoxanide, which undergoes glucuronidation and urinary elimination with an elimination half-life of roughly 7 h; tizoxanide is highly bound (>99%) to plasma proteins; need for dose adjustments uncertain	Gastrointestinal effects, respiratory distress

*Resistance in seasonal influenza A H3N2 and 2009 pandemic influenza A H1N1; avian influenza A H7N9, A H10N8, and A H9N2; and some influenza A H5N1 viruses. †Neuraminidase inhibitors prevent destruction of sialic-acid-bearing receptors recognised by influenza A and B virus haemagglutinins. This action blocks virus from being released from infected cells and spreading through respiratory secretions to initiate new cycles of replication. Neuraminidase inhibitors might also inhibit virus binding to cells. ‡Except seasonal influenza A H1N1 during 2007–09. §Approved in China, Japan, and South Korea. ¶Approved in Japan. ‖Approved in Japan for treatment of novel or re-emerging influenza virus infections (restricted to cases in which other anti-influenza drugs are ineffective or not sufficiently effective).

***Table 1*: Influenza antivirals approved or in advanced clinical development**

combination with other adjunct interventions, such as immunomodulators and host-directed therapies.

Influenza viruses

Drugs

Two classes of antiviral drugs are approved for the prevention and treatment of influenza in most countries: M2 inhibitors (amantadine and rimantadine) and neuraminidase inhibitors (oseltamivir, peramivir, zanamivir, and laninamivir; table 1).[12–43] In general, antiviral treatment is indicated as early as possible for any patient with confirmed or suspected influenza who has severe, complicated, or progressive illness or is admitted to hospital, and in outpatients at higher risk of influenza complications.[12,13] Time to treatment after onset of symptoms, illness severity, and extent of viral replication are key variables with respect to response. Starting of treatment should not be delayed for diagnostic testing. M2 inhibitors—also known as adamantanes—are ineffective against influenza B viruses and recently circulating influenza A H3N2 and 2009 pandemic influenza A H1N1 viruses, which are resistant because of an S31N mutation in the M2 ion channel.[12] However, a proportion of avian influenza A H5N1 strains will be susceptible,[14] and the combined use of an adamantane and a neuraminidase inhibitor improves antiviral activity for susceptible isolates.[15]

Two neuraminidase inhibitors are approved for use in most countries: oseltamivir and zanamivir. Laninamivir is approved for use in Japan only, and peramivir in China, Japan, and South Korea. Several observational studies have shown that when adults admitted to hospital with severe influenza are given oseltamivir, mortality falls and clinical outcomes improve, especially when treatment is initiated within 2 days of the onset of symptoms (but positive effects are noted when it is begun as late as 4–5 days after onset).[12,13,16,17] Oseltamivir reduces mortality in influenza A H5N1 infection when given before the onset of respiratory failure,[18] and might be beneficial when started as late as 6–8 days after symptom onset.[19] In patients admitted to hospital with severe influenza A H7N9 infection, reduction of viral load after treatment with oseltamivir correlated with improved outcome, whereas the emergence of virus resistant to neuraminidase inhibitors that harbours an Arg292Lys substitution is associated with poor outcomes and poor response to oseltamivir and peramivir.[20]

The standard duration of oseltamivir treatment is 5 days; longer treatment is recommended for critically ill patients with respiratory failure, who often have prolonged viral replication in the lower respiratory tract despite treatment.[13] Whether increased doses provide greater antiviral effects in such patients is under investigation. A randomised controlled trial[21] of patients in hospital (76% of whom were children) showed no virological or clinical advantages when a double dose of oseltamivir was given rather than a standard dose. No additional benefit was noted with high-dose oseltamivir in adults admitted with influenza A, although a faster virological response was noted in those with influenza B.[22] However, in a randomised controlled trial[23] of 18 critically ill patients with 2009 pandemic influenza A H1N1, a triple-dose oseltamivir regimen was associated with significantly higher proportions of viral clearance at 5 days than was standard therapy (78% *vs* 11%; p=0·015).[23] Studies of intravenous neuraminidase inhibitors that are underway should provide further data on the value of high-dose therapy.

Zanamivir and laninamivir have generally similar profiles of susceptibility. For example, the His275Tyr mutation confers high-level resistance to oseltamivir carboxylate and reduced susceptibility to peramivir in N1-containing viruses but does not substantially diminish susceptibility to zanamivir and laninamivir.[30] Inhaled zanamivir has not been studied in detail in severely ill patients or those admitted to hospital, in whom effective delivery to sites of viral replication and tolerability could be an issue. By contrast, intravenous zanamivir has been used widely on a compassionate basis since the 2009 H1N1 pandemic, particularly for late treatment of critically ill adults with 2009 pandemic influenza A H1N1 virus infection and those with suspected or proven oseltamivir resistance.[31] One trial[32] has shown no drug-related trends in safety measures, and a subset of 93 patients positive at baseline for influenza showed a median decrease in nasopharyngeal viral RNA load of 1·42 $\log_{10}$ copies per mL after 2 days of treatment. A phase 3 trial in patients who have been admitted to hospital is underway (NCT01014988). A phase 2 randomised controlled trial of inhaled laninamivir in uncomplicated influenza failed to show superiority in illness alleviation (primary endpoint) compared with placebo. The trial, involving 639 patients, tested 40 mg and 80 mg doses of the inhaled drug. The median time to alleviate flu symptoms was 102·3 h for the 40 mg dose and 103·2 h for the 80 mg dose, compared with 104·1 h for the placebo (NCT01793883).

DAS181 has host-directed receptor-destroying action, which is inhibitory for parainfluenza and influenza viruses, including those resistant to aminoadamantanes and neuraminidase inhibitors.[15] When delivered topically, it is effective in animal models of lethal influenza caused by the H5N1 and H7N9 viruses, including the neuraminidase-inhibitor-resistant Arg292Lys-containing variant.[35] In a phase 2 randomised controlled trial,[36] inhaled DAS181 reduced pharyngeal viral replication in uncomplicated influenza but did not reduce nasal viral loads or improve clinical outcomes. Case reports[37] suggest that inhaled or nebulised DAS181 might be effective in immunocompromised hosts with severe parainfluenza lung disease.

Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) is active against influenza A, B, and C viruses, including strains resistant to approved antivirals, and a broad range of other RNA viruses when given at

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	Study type	Target population	Combination (number treated)	Comparator (number treated)	Outcomes/comments
Ison et al[52]	Double-blind RCT	Adults in hospital with influenza-associated lower respiratory tract illness	Oral rimantadine and nebulised zanamivir (20)	Oral rimantadine and nebulised saline (21)	Post-hoc analysis showed faster cough resolution but no significant differences in the proportion of patients shedding virus by treatment day 3 (57% zanamivir plus rimantadine, 67% placebo plus rimantadine), or in the durations of hospitalisation and supplemental oxygen use Underpowered because of low enrolment
Duval et al[52]	Double-blind RCT	Adult outpatients with uncomplicated seasonal influenza	Oral oseltamivir and inhaled zanamivir (157)	Oral oseltamivir (141) or inhaled zanamivir (149)	Slower virological and clinical responses in those given combined therapy compared with those given oseltamivir alone
Kim et al[53]	Retrospective, observational	Critically ill patients with 2009 pandemic influenza A H1N1	Oral amantadine, ribavirin, and oseltamivir (24)	Oral oseltamivir (103)	Non-significant trends towards lower 14 day (17% *vs* 35%, p=0·08) and 90 day (46% *vs* 59%, p=0·23) mortality in combination recipients than in those receiving oseltamivir alone No virology data
Hung et al[47]	Prospective, observational	Critically ill patients with 2009 pandemic influenza A H1N1	Convalescent plasma and oral oseltamivir (20)	Oral oseltamivir (73)	Crude mortality in the plasma group significantly lower than that in the control group (20·0% *vs* 54·8%, p<0·01) Faster nasopharyngeal viral clearance Plasma with neutralising antibody titre of ≥1/160
Hung et al[48]	Double-blind RCT	Critically ill patients with 2009 pandemic influenza A H1N1	Hyperimmune intravenous immunoglobulin from convalescent plasma and oral oseltamivir (17)	Intravenous immunoglobulin manufactured before 2009 (18)	Subgroup of 12 patients treated with hyperimmune intravenous immunoglobulin within 5 days of symptom onset had a lower viral load and reduced mortality (0% *vs* 40%; odds ratio 0·14 [95% CI 0·02–0·92], p=0·04) than did the 10 given given control intravenous immunoglobulin No overall difference in mortality (29% *vs* 23%)
Wang et al[54]	Open-label RCT	Critically ill patients with 2009 pandemic influenza A H1N1	Sirolimus, oseltamivir, and corticosteroids (19)	Oseltamivir and corticosteroids (19)	More rapid improvement in partial pressure of oxygen, fraction of inspired oxygen, and sequential organ failure assessment scores; shorter ventilator use (median 7 days *vs* 15 days, p=0·03); and faster viral clearance in the sirolimus than in the control group

RCT=randomised controlled trial.

***Table 2*: Representative clinical effectiveness studies of combination influenza therapeutics, by study**

somewhat higher concentrations.[38] Combinations of favipiravir and neuraminidase inhibitors have additive and synergistic effects in preclinical models,[39] but clinical trials have been restricted to uncomplicated influenza so far. These clinical trials (combination amantadine, ribavirin, and oseltamivir *vs* oseltamivir monotherapy [NCT01227969], nitazoxanide *vs* oseltamivir *vs* combination *vs* placebo [NCT01610245], favipiravir *vs* placebo randomised controlled trial in outpatients [NCT02008344, NCT2026349]), which have not been published, suggest that favipiravir has antiviral effects similar to those of oseltamivir.[40] A randomised controlled trial[41] showed that favipiravir shortened the time to alleviation of influenza symptoms by about 15 hours compared with placebo, and further studies are underway.

Nitazoxanide is an oral antiparasitic drug with immunomodulatory effects, including upregulation of interferon and various interferon-inducible genes and a specific influenza-inhibitory effect related to blockade of haemagglutinin maturation.[42] Nitazoxanide inhibits influenza replication in vitro[43] and in a phase 2 randomised controlled trial[44] had significant antiviral effects (1·0 $\log_{10}$ reduction in nasal viral loads) and resulted in a significantly faster time to alleviation of illness (roughly 20 h difference in medians from placebo) in uncomplicated influenza.[44] A placebo-controlled randomised trial of nitazoxanide versus oseltamivir—and the combination thereof—in uncomplicated influenza and a hospital-based study of its use in severe respiratory illness are in progress (NCT01610245).

Immune-based treatments for influenza

Non-randomly assigned studies and case reports suggest that convalescent plasma with neutralising antibodies is a useful add-on therapy for patients with SARS and severe influenza pneumonia, including that caused by influenza A H5N1.[45] A recently published systematic review of available SARS and influenza treatment studies employing convalescent plasma or serum found a significant overall mortality benefit.[46] A prospective observational study[47] showed lower crude mortality and faster nasopharyngeal viral clearance in plasma-treated patients who were admitted with severe 2009 pandemic influenza A H1N1 infection, whereas in a randomised controlled trial[48] a reduction in mortality was reported in severe illness when hyperimmune globulin was given within 5 days of the onset of symptoms (table 2). Heterosubtypic haemagglutinin stem-neutralising antibodies, which are highly effective in animals,[49] are entering clinical evaluation in human beings.

Combinations of antivirals

The combination of antivirals with different mechanisms of actions (eg, a neuraminidase inhibitor with a polymerase inhibitor such as favipiravir,[38] a broad-spectrum anti-haemagglutinin-neutralising antibody,[49] or nitazoxanide) for the management of severe forms of influenza or infections in immunocompromised hosts is the subject of ongoing study. The use of various antiviral drug combinations to improve antiviral potency, reduce

the emergence of resistance, and perhaps spare doses has been explored in preclinical studies.[15,50] However, few combination studies have been focused on effectiveness (table 2). By contrast with combinations of drugs with differing mechanisms of action, combination of optimum doses of drugs with similar mechanisms of action (eg, dual neuraminidase inhibitors) does not enhance[51] antiviral activity and can sometimes result in antagonism.[52,53] A triple combination of antiviral drugs consisting of amantadine, ribavirin, and oseltamivir showed synergistic in-vitro activity against influenza A viruses that were susceptible to all three drugs and also those resistant to the amantadine or oseltamivir at baseline, including 2009 pandemic influenza A H1N1 virus.[51] In a retrospective study[53] of critically ill adults, mortality rates did not differ between those who received a triple combination of antiviral drugs and those receiving oseltamivir only, and a randomised controlled trial sponsored by the National Institute of Allergy and Infectious Diseases in higher-risk outpatients is underway (NCT01227969).

Antivirals combined with host-directed therapies

Host-directed therapies aim to reduce the damaging consequences of the host immune response to the pathogen. Combinations of antivirals with host-directed therapies such as the immunomodulator sirolimus, an mTOR inhibitor that blocks host pathways needed for viral replication (table 2),[54] might also enhance antiviral activity. Other host-directed therapies inhibiting cellular targets needed for efficient viral replication (eg, the Raf–MEK–ERK mitogenic kinase cascade and the IKK–NF-κB module) might provide future options for clinical testing.[15]

The role of adjunctive immunomodulatory therapies in severe influenza and other respiratory viral infections remains uncertain. Several observational studies show that systemic corticosteroids given for 2009 pandemic influenza A H1N1-associated viral pneumonia increased the risk of mortality and morbidity (eg, secondary infections), especially when there was a delay in initiation, or absence of, effective antiviral therapy.[45] Their use might delay viral clearance and increase the risk of the emergence of resistance[20] and fungal infections.[45]

Other potential adjunctive therapies for influenza include intravenous immunoglobulin, N-acetylcysteine, statins, macrolides, peroxisome proliferator-activated receptor agonists, celecoxib, mesalazine, plasmapheresis, and haemoperfusion.[45] Chloroquine was effective against influenza A H5N1 infection in one animal model[55] but was ineffective in other animal models and one human randomised controlled trial.[56,57]

MERS-CoV infection

Interferons

MERS-CoV infection can cause severe respiratory disease, and has higher mortality in those with medical comorbidities. Although empirical treatment with a range of antivirals has been tried for severe respiratory tract infections caused by MERS-CoV and SARS-CoV, no regimens have been rigorously assessed in clinical trials (panel).[58,59] MERS-CoV elicits attenuated innate immune responses with delayed proinflammatory cytokine induction in cell culture and in vivo.[60,61] It is also readily inhibited by type 1 interferons (interferon alfa and especially interferon beta), suggesting a potential therapeutic use for interferons. Early pegylated interferon alfa therapy was effective in a SARS primate model, and treatment with interferon-alfa-consensus-1 plus systemic corticosteroids was associated with improved oxygen saturation and more rapid resolution of radiographic lung opacities than were systemic corticosteroids alone in an uncontrolled study of patients with SARS patients.[62] Further studies of interferons in MERS-CoV seem warranted.

***Panel*: Potentially useful antiviral agents for Middle East respiratory syndrome coronavirus (MERS-CoV) infection**

- Neutralising antibody
  - Convalescent plasma
  - Polyclonal human immunoglobulin from transgenic cows
  - Equine $F(ab')_2$ antibody fragments, camel antibodies
  - Anti-S monoclonal antibodies
- Interferons
  - Interferon alfa
  - Interferon beta
- Repurposed drugs
  - Ribavirin (with or without interferon)
  - HIV protease inhibitors (lopinavir, nelfinavir)
  - Cyclophilin inhibitors (ciclosporin, alisporivir)
  - Chloroquine (active in vitro)
  - Mycophenolic acid
  - Nitazoxanide
- Recombinant human mannose-binding lectin
- siRNA to key MERS-CoV genes

Antiviral drugs

Ribavirin was used extensively in patients with SARS without any beneficial effects and was complicated by haemolytic anaemia and metabolic disturbances in many cases.[58,59] A combination of interferon alfa 2b and ribavirin reduced lung injury and moderately decreased viral replication (<1·0 $\log_{10}$ reduction in lung titres) when given to rhesus macaques within 8 h of inoculation with MERS-CoV.[63] The treatment combination was given to several severely ill patients with MERS, but the infections proved fatal, probably because of late administration in the advanced stage of the disease.[64,65] Ribavirin has in-vitro inhibitory effects against MERS-CoV.[66,67] The inhibitory concentrations of ribavirin are very high for MERS-CoV and exceed those

that can be achieved with usual dosing regimens, except possibly peak concentrations after high intravenous doses.

The use of protease inhibitors with lopinavir and ritonavir as initial therapy in SARS was associated with significantly less death (2·3% *vs* 15·6%, p<0·05) and intubation (0% *vs* 11·0%, p<0·05) than was use of ribavirin alone in a matched historical cohort (n=44 for lopinavir and ritonavir as intial treatment *vs* n=634 for the matched historical cohort).[68] However, one study reported that nelfinavir and lopinavir have high 50% effective inhibitory concentrations (EC_{50}) against MERS-CoV in vitro,[66] whereas another found inhibition with lopinavir at clinically achievable concentrations.[69]

Immunomodulatory and immune-based therapies

Several drugs have shown inhibitory effects against MERS-CoV in cell cultures, including interferons, ciclosporin, and mycophenolic acid.[66,67,69] Mycophenolic acid was inhibitory at clinically achievable concentrations, and the combination of mycophenolic acid and interferon β1b lowered the EC_{50} of each drug by one-to-three times.[66] Dipeptidyl peptidase 4 (DPP4), also known as CD26, is a functional receptor for MERS-CoV, and an anti-CD26 polyclonal antibody showed in-vitro inhibitory effects on MERS-CoV.[70] By contrast, inhibitors of the enzymatic action of DPP4 (eg, gliptins) did not inhibit viral replication.

Timely administration of neutralising antibodies could have a high likelihood of therapeutic success.[46] Treatment with convalescent plasma (from patients who have recovered from SARS-CoV infection) containing high levels of neutralising antibody within 2 weeks of illness onset resulted in a higher proportion of discharges at day 22 than did treatment more than 14 days after onset (58% *vs* 16%, p<0.001).[71] Some patients who survived MERS-CoV infection had high concentrations of neutralising antibody,[72,73] and convalescent plasma, if available, might provide a good treatment option for other severe cases.

Systemic corticosteroids have been used empirically frequently in SARS[58,59] and MERS-CoV[64,65] infections to dampen immunopathological host responses. However, survival benefit is unclear,[59,64,65] and a randomised controlled trial[74] done in Hong Kong showed that systemic corticosteroids could delay viral clearance in SARS. A retrospective analysis[75] showed worse outcomes when systemic corticosteroids were given in SARS. Consequently, their use should be avoided unless a carefully controlled prospective study is done to test their effectiveness when combined with an antiviral. Several observational studies have shown that systemic corticosteroids given for 2009 pandemic influenza A H1N1-asssociated viral pneumonia or acute respiratory distress syndrome increased the risk of mortality and morbidity (eg, secondary bacterial or fungal infections), especially if there is delay or lack of effective antiviral therapy.[45] Use of systemic corticosteroids has probably contributed to delayed viral clearance and emergence of antiviral resistance in patients with severe influenza A H7N9 infection requiring extracorporeal membrane oxygenation.[20] Influenza increases the risk of invasive aspergillosis, especially among immunocompromised patients, and this is often a silent infection in the early stages,[76] so direct surveillance with aspergillus antigen and PCR testing on respiratory secretions is advisable. Patients treated for fungal infections will have to undergo antifungal therapeutic drug monitoring.[77]

Data are insufficient to support routine use of any of the immune therapies. Better animal data and careful systematic clinical studies, including serial virological measurements of priority treatments such as convalescent plasma and interferons (and randomised controlled trials if case numbers are sufficient), are needed. Currently, clinical management of patients with severe respiratory tract infections due to MERS-CoV largely relies on meticulous intensive care supportive treatment and prevention of complications.

Host-directed therapy for viral infections

T-cell therapy

Research done in patients with haemopoietic stem-cell transplants shows that adoptive transfer of antigen-specific T cells can restore protective immunity and prevent or reverse disease due to opportunist viral infections such as cytomegalovirus.[78] In transplant recipients, transfer of donor-derived T cells can result in resolution of infection through expansion of virus-specific T cells, with associated clinical improvement.[79] Transfer of donor T cells is associated with the risk of severe acute graft-versus-host disease, and thus most T-cell therapies have been done in patients who have low lymphocyte counts. Lymphopenia enables only a very low number of T cells to be transferred, which then proliferate in lymphopenic hosts, most likely as a result of the interleukins 7 and 15 if the patient does not receive immunosuppressive treatment during T-cell therapy.[80]

T-cell therapy targeting cytomegalovirus strains resistant to drug treatment is clinically relevant in lung transplant recipients.[81] T-cell expansion requires time to induce clinical regression of viral infection. Several other approaches might be applicable in situations that necessitate fast clinical action—eg, use of synthetic MHC antigens loaded with the relevant peptide from the pathogen of interest (so-called tetramer or multimer MHC–peptide complexes), which engage pathogen-specific lymphocytes expressing the pathogen-specific T-cell receptors. Pathogen-specific T cells can be isolated through use of soluble MHC–peptide complexes, and can immediately be transferred into patients for salvage treatments for viral infections.[82] T-cell expansion can also be achieved with several stimuli targeting several infectious pathogens.[83] Expansion of T cells targeting several antigens of cytomegalovirus, Epstein-Barr virus, and adenovirus provides broad antiviral specificity after stem-cell transplantation.[84] An alternative approach to

become independent of ex-vivo expansion of T cells is the identification of T-cell receptors that would recognise viral infected cells that could be transferred into recipient effector cells.[85] T cells can also be engineered to produce an antiviral RNA that would block viral infection.[86]

Antisense molecules

Synthetic antisense molecules, such as phosphorodiamidate morpholino oligomers, are structurally similar to RNA but the phosphorodiester linkage is replaced with a neutral phosphorodiamidate linkage and the ribose ring with a six-membered morpholino ring.[87] They change gene expression by inhibiting translation, disrupting RNA secondary structure, and interfering with pre-mRNA splicing.[88] The usefulness of phosphorodiamidate morpholino oligomers coupled to arginine-rich cell-penetrating peptides has been repeatedly demonstrated against bacterial pathogens[89] and could be a viable option for any microbial gene of interest.

Specific antibody therapy

Specific biological therapy for infectious pathogens targets not only drug-resistant pathogens but also their immune evasion mechanisms.[90] An antibody directed against CD19 (a B-cell marker) fused to a T-cell signalling molecule can be expressed in T cells and could kill target cells once they encounter their nominal target antigen. Such CD19 chimeric-antigen-receptor cells are used to remove Epstein-Barr-virus-positive lymphoma cells in the case of post-transplantation proliferative diseases.[91] Similar approaches can be used for the effective removal of pathogen-infected cells when very specific antibodies exist and if target molecules are expressed on infected cells only.[92]

Antibiotic-resistant bacterial respiratory tract infections

The frequency and spectrum of resistance to antibiotics in specific bacterial pathogens that cause respiratory tract infections continues to increase worryingly. Multidrug-resistant *Streptococcus pneumoniae*—with resistance to three or more antibiotics—was initially noted in 1977 in South Africa[93] and subsequently in many other countries, with alarming rates of 30–50% of *S pneumoniae* that are multidrug resistant in the USA and Spain.[94–96] The European Antimicrobial Resistance Surveillance System showed that 22·2% of *S pneumoniae* were intermediate penicillin susceptible, 10·9% were penicillin resistant, and 21·1% were resistant to erythromycin.[97]

Concerns about multidrug-resistant and pan-antibiotic-resistant Gram-negative bacteria[98,99] are focused on *Klebsiella pneumoniae*, *Enterobacter* spp (production of extended spectrum β lactamase, *Klebsiella pneumoniae* carbapenemase, NDM1, and AmpC), *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. In one survey of US health centres, 78% of Gram-negative bacteria were resistant to all antibiotics except colistin (to which 62% of *Acinetobacter* spp, 59% of *Pseudomonas* spp, and 52% of *Enterobacter* spp were resistant).[98] Therapeutic options to treat these infections are limited.[100,101]

Carbapenems are recommended for organisms that produce extended-spectrum β lactamases.[101] In a meta-analysis,[102–106] doripenem was more effective for *P aeruginosa* infections than were comparators in a modified intention-to-treat analyses. Polymyxin B and colistin are concentration-dependent bactericidal agents that bind to bacterial cell membranes and have reliable activity against *Acinetobacter* spp. Novel β-lactamase inhibitors[107] and antibiotic combination therapies[108] might provide stopgap measures for fulfilling clinical need. Antibiotic development pipelines remain thin,[109,110] and global attention is focused on increasing awareness for investments into the development of new antibacterial agents[111] and other antibacterial innovations, coupled to raising global awareness for more prudent use of available drugs.[112]

Multidrug-resistant pulmonary tuberculosis

Incidence

In 2012, an estimated 1·3 million people died worldwide from tuberculosis, 170 000 of whom had multidrug-resistant disease.[113] Multidrug-resistant tuberculosis, which is caused by *Mycobacterium tuberculosis* bacilli resistant to at least isoniazid and rifampicin, is now widespread globally, with an estimated half a million cases in 2012.[2] Extensively drug-resistant tuberculosis—resistance to rifampicin, isoniazid, any fluoroquinolone, and at least one of the three injectable second-line drugs, amikacin, kanamycin, and capreomycin—has been reported in 92 countries.[113] WHO recommends use of second-line drugs for 18–24 months or longer for extensively drug-resistant or multidrug-resistant disease.[114,115] Treatment success rates are low in both individualised and standard regimens and new drugs and regimens are needed.

New drugs pipeline

In the past 5 years, a promising pipeline of new drugs for the treatment of multidrug-resistant and extensively drug-resistant tuberculosis has emerged.[115] Progress has been made by repurposing drugs that are already available, including re-engineering existing antibacterial compounds and redesigning scaffolds, leading to discovery of new compounds.[116,117] Two new drugs, delamanid (OPC-67683) and bedaquiline (TMC207 or R207910), have been approved by regulatory authorities. These new drugs are combined with older drugs to treat multidrug-resistant disease.[118,119]

Host-directed adjunct therapies

Several approaches to rational development of adjunct immune-based therapies for multidrug-resistant tuberculosis have been developed.[120,121] Non-steroidal anti-inflammatory drugs can reduce *M tuberculosis* load and

	Aspergillosis			*Pneumocystis jirovecii* pneumonia
	Invasive	Chronic	Allergic bronchopulmomnary aspergillosis	
Incidence (per 100 000)	8·6	10·4	Unknown	5·6
Prevalence (per 100 000)	..	32·8	286	..
Global burden[131]	~200 000	~3 000 000	4 800 000	~400 000
Untreated mortality	~100%	~30%*	<1%	100%
Treated mortality	30–90%	~10%*	<1%	10–20% or 50%†

Severe asthma with fungal sensitisation is not included. Annual incidence and prevalence quoted for aspergillosis refer to European data; those for *P jirovecii* pneumonia are global data. *12 month mortality. †Mortality is lower in patients with AIDS than in other immunocompromised patients.

***Table 3*: Frequency and mortality of common fungal pulmonary infections**

alleviate lung disease[122] in mice.[123] Efflux pump inhibitors such as verapamil and reserpine reduce macrophage-induced drug tolerance, and thus could be used as adjunct host-directed therapies.[124,125] Phosphodiesterase inhibitors such as cilostazol and sildenafil improve mycobacterial clearance and decrease time to sterilisation by reducing tissue inflammation.[126]

A range of adjunct immunotherapy approaches implicating cytokines or their inhibitors and other biological immunomodulatory compounds are being assessed as means to limit damage from inflammatory responses against *M tuberculosis*. Various cytokine regimens, including interferon c or interleukin 2, have been assessed, with variable effect.[127,128] The anti-inflammatory effects of macrolide antibiotics need to be further studied.[129] Whole genome sequencing might allow for rapid determination of resistance patterns of *M tuberculosis* strains, enabling tailored treatment regimens. Other immunomodulatory strategies include restoration of effective antipathogen-directed immuno-responses—and consequent decreasing of damaging host responses in lung tissues—in multidrug-resistant tuberculosis with infusions of the patient's own bone-marrow-derived stromal cells. A phase 1 trial showed that the procedure is safe,[130] and phase 2 trials are planned to assess the effects of mesenchymal stromal cell adjunct therapy on clinical and microbiological outcomes.

Fungal respiratory tract infections

Frequency

Invasive fungal respiratory tract infections are increasingly reported worldwide (table 3).[131,132] The two most common pulmonary fungal pathogens are *Aspergillus fumigatus* and *Pneumocystis jirovecii*. They increasingly represent primary causes of morbidity and mortality in critically ill patients across Europe, Africa, and Asia as a result of more people living with HIV, increased use of immunomodulatory drugs in patients with cancer, transplantations, and use of broad-spectrum antibiotics. Some patients with relapsed or microbiologically unconfirmed multidrug-resistant tuberculosis have alternative diagnoses, including chronic pulmonary aspergillosis, and more comprehensive searches for alternative fungal diagnoses in smear and culture negative cases should be done in patients with multidrug-resistant disease.[133]

Invasive pulmonary aspergillosis

Aspergillus is the most important fungal cause of invasive pulmonary disease, and *A fumigatus* is the cause in more than 75% of cases. Voriconazole is the most effective treatment for invasive aspergillosis but resistance has been noted on all continents except South America.[134,135] Widespread use of the azoles as fungicides in agriculture has led to the environmental development of pan-azole resistance.[136] Resistance can also emerge during treatment, typically to itraconazole, and is possibly linked to a combination of low blood concentrations of the drug and high fungal loads.[137–139]

Modelling suggests that more than 6·5 million people have severe asthma with fungal sensitisations, as much as 50% of adults with asthma who attend secondary care have fungal sensitisation, and an estimated 4·8 million adults have allergic bronchopulmonary aspergillosis.[140,141] People with asthma who are sensitised to *A fumigatus* have a much higher rate of bronchiectasis than do those who are unsensitised. Reclassification of aspergillosis in adults with cystic fibrosis by aspergillus serology (IgE and IgG) and both PCR and antigen on sputum showed three distinct classes of aspergillosis. 18% had allergic bronchopulmonary disease, 15% had aspergillus sensitisation, and 30% had aspergillus bronchitis; the remaining patients had no disease. Long-term oral antifungal therapy is beneficial for 60–80% of patients with asthma, but is of unproven benefit in cystic fibrosis.[142] Resistance in *A fumigatus* has been reported throughout Europe in roughly 4% of samples from patients with cystic fibrosis.[143,144]

Disseminated *Emmonsia* spp infections

A new fungus causing disseminated infections in patients with AIDS was identified in 2009.[145] Molecular identification on the basis of ITS1 and ITS2 sequencing showed that all isolates of this new species were tightly clustered and were most similar to *Emmonsia pasteuriana* and *Emmonsia parva*, and slightly more distantly related to *Histoplasma capsulatum*. Clinical features of infection included fever, loss of weight, anaemia, skin lesions akin to those in disseminated histoplasmosis, and a chest radiograph similar to that noted in pulmonary tuberculosis. The fungus was cultured from skin and blood, but not sputum or CSF. Significant clinical responses were noted when patients were given intravenous amphotericin B followed by itraconazole.[145]

Advances in antifungal therapy

A large combination study[146] of voriconazole and anidulafungin for invasive aspergillosis in 177 patients did not reach its primary endpoint of reduced mortality,

	Manufacturer	Spectrum	Route	Mode of action	Trial stage	Comments
Albaconazole	Actavis	Broad spectrum	Intravenous, oral	14 α-demethylase inhibitor	2	Vulvovaginal candidiasis and onychomycosis
Scy078 (MK-3118)	Scynexis	Broad spectrum	Intravenous, oral	Glucan synthase inhibitor	2a	Phase 2 development on candidiasis
VT1161/1129	Viamet	*Candida* spp, dermatophytes, *Cryptococcus* spp	Oral, topical	14 α-demethylase inhibitor	2	Phase 2 trials ongoing for vulvovaginal candidiasis and tinea pedis
MGCD290	Mirati Therapeutics	*Candida* spp	Oral	Histone deacetylase inhibitor	2	Targets vulvovaginal candidiasis; potentiator of azoles
Nikkomycin Z	University of Arizona	*Coccidioides* spp	Oral	Chitin synthesis inhibitor	2	Phase 2 studies expected to start 2014–15
T-2307	Toyama	Broad spectrum	Intravenous, oral	Mitochondrial polyamine transport inhibitor	1	Focused on oesophageal and invasive candidiasis
F901318	F2G	Moulds	Intravenous, oral	Novel, not disclosed	1	Aspergillosis, other mould infections

***Table 4*: New antifungal drug pipeline**

although patients with positive galactomannan seemed to benefit most. Guidelines for management of invasive aspergillosis still favour voriconazole over all other treatments and combination therapy is not usually recommended. A tablet formulation of posaconazole, which is more bioavailable than the oral suspension, is available and can be given once a day,[147] and the US Food and Drug Administration has approved an intravenous suspension of the drug. The only new drug to be approved is isavuconazole, a broad-spectrum azole, which will be available in intravenous and oral forms (application for approval was submitted in July, 2014). Itraconazole seems safe in the first trimester of pregnancy, whereas fluconazole increases the risk of Fallot's tetralogy by a factor of three to one in 1000.[148]

Drivers for the development of new antifungal drugs include inadequate response rates, the absence of oral preparations of echinocandins, drug interactions, important drug toxic effects (especially amphotericin B and voriconazole), and triazole and echinocandin resistance. Several drugs are being repurposed for use as antifungals, and new drugs are under development (table 4).[149–155] Sertraline, which is used for depression, has synergistic activity with fluconazole in a murine model of cryptococcal infection.[156] Calcineurin and targets of rapamycin inhibitors have antifungal activity, which is synergsitic with that of azoles.[157] Hsp90 inhibitors initially developed for cancer treatment can improve fluconazole activity in vitro and in animals.[158] Enoxacin, a fluoroquinolone antibiotic, shows activity in a murine candidiasis model.[159]

Host-directed therapy

Although azoles are important for the treatment of invasive pulmonary aspergillosis, the degree of immunosuppression and other immunological factors have a role in treatment outcomes. Antifungal immune responses could be improved by adaptive transfer of pathogen-specific T cells directed against invasive and pulmonary fungal infections, particularly infections with candida, aspergillus, and mucormycetes, especially after allogeneic stem-cell transplantation. T-cell responses are MHC class I restricted (for CD8-positive T cells) or MHC class II restricted (for CD4-positive T cells), and thus an effective T-cell response needs to match the genetic background of the patient. T-cell transfer was developed on the basis of the promising finding that transfer of pathogen-specific T-cell clones induces clinically significant responses.[160,161] Several approaches have been used to obtain these pathogen-specific T cells. Anti-pathogen-specific T cells can be expanded ex vivo under appropriate conditions (usually with the help of recombinant cytokines, synthetic peptides, or cellular components representing the pathogen). Responder T cells are identified by interferon-γ production, removed via an interferon-capture assay, and transferred into the patient. This approach requires time for expansion of T cells (either the patient's own or those of an MHC-matched donor). This protocol enabled the expansion of *Aspergillus* spp, *Candida* spp, and *Mucor* spp-reactive T cells defined by interferon-γ production. Upon re-encounter with the

Search strategy and selection criteria

We searched for publications in English on PubMed (from Jan 1, 1970, to June 30, 2014), Google Scholar (from Jan 1, 1970, to Aug 4, 2014), the Cochrane Library (from Jan 1, 2001, to June 30, 2014), and Embase (from Jan 1, 2001, to Aug 4, 2014) with the terms "respiratory tract", "pneumonia", "infections", "bacteria", "virus", "fungus", and "mycobacteria". We also combined these terms with the words "antibiotic", "antibiotic resistance", "treatment", "drugs", "drug development", "drug pipeline", "antibiotic development", "host-directed", "therapy", "adjunct therapy", "steroids", and "immunotherapy". We complemented the search with publications from WHO, the US Centers for Disease Control and Prevention, http://clinicaltrials.gov, and Google Scholar. We also reviewed studies cited by articles identified by this search.

nominal target antigen, the T cells proliferated and increased the antifungal reactivity of phagocytes.[162]

Conclusion

New and antimicrobial-resistant species of bacteria, viruses, and fungi continue to emerge because of the remarkable genetic and adaptable plasticity of the microbiota.[163] Respiratory tract infections are among the top two causes of death globally.[164,165] Microorganisms do not respect international boundaries, and ease of travel and airborne spread make them a threat to global health security. The increasing frequency of antibiotic resistance and limited therapeutic options emphasise the urgent need for more international cooperation to tackle new emerging microbial threats and multidrug-resistant microbes. Development of new therapeutic options needs to be coupled to international regulations on the use and prescription of antimicrobial drugs.

Contributors

DSH and AZ coordinated the writing of this Series paper and wrote the draft outline, and subsequent and final drafts. All authors contributed relevant text and tables on their expert sections or sections and contributed to finalising the paper.

Declaration of interests

FGH has served as non-paid consultant for multiple companies engaged in marketing and/or clinical development of antivirals for respiratory viral infections including several whose therapeutics are discussed in this review (Adamas, Biocryst, GSK, Genentech, Janssen, Roche, Romark, Toyama/Medivector, Visterra). DWD holds founder shares in F2G, a University of Manchester spin-out company. He acts as a consultant to Trinity Group, T2 Biosystems, GlaxoSmithKline, Sigma Tau, Oxon Epidemiology, and has consulted for Merck and Astellas and he has been paid to give talks on behalf of Astellas, Gilead, and Pfizer. All other authors declare no conflicts of interest.

Acknowledgments

AZ acknowledges support from the European Union FW7 Rid-RTI programme grant; European Developing Countries Clinical trials Partnership (EDCTP) TB NEAT, PANACEA, and REMox grants; UBS Optimus Foundation, Switzerland; and National Institute for Health Research Biomedical Research Centre, University College London Hospital, London, UK. DWD receives grant support from the National Institute of Allergy and Infectious Diseases, National Institute of Health Research, the European Union, and the Fungal Infection Trust. MM acknowledges support from the Swedish Heart and Lung Foundation, Vinnova, Vetenskapsrådet (Swedish Research Council), and EDCTP. Adam Zumla, UCL School of Pharmacy, kindly provided technical and administrative support.

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