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ORIGINAL

INHALE WP3, a multicentre, open-label, pragmatic randomised controlled trial assessing the impact of rapid, ICU-based, syndromic PCR, versus standard-of-care on antibiotic stewardship and clinical outcomes in hospital-acquired and ventilator-associated pneumonia

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Abstract

Purpose: INHALE investigated the impact of seeking pathogens by PCR on antibiotic stewardship and clinical outcomes in hospital-acquired and ventilator-associated pneumonia (HAP and VAP).

Methods: This pragmatic multicentre, open-label RCT enrolled adults and children with suspected HAP and VAP at 14 ICUs. Patients were randomly allocated to standard of care, or rapid in-ICU syndromic PCR coupled with optional prescribing guidance. Co-primary outcomes were superiority in antibiotic stewardship at 24 h and non-inferiority in clinical cure of pneumonia 14 days post-randomisation. Secondary outcomes included mortality, ICU length of stay and evolution of clinical scores.

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Results: 554 eligible patients were recruited from 5th July 2019 to 18th August 2021, with a COVID-enforced pause from 16th March 2020 and 9th July 2020. Data were analysed for 453 adults and 92 children (68.4% male; 31.6% female). ITT analysis showed 205/268 (76.5%) reviewable intervention patients receiving antibacterially appropriate and proportionate antibiotics at 24 h, versus 147/263 (55.9%) standard-of-care patients (estimated difference 21%; 95% CI 13–28%). However, only 152/268 (56.7%) intervention patients were deemed cured of pneumonia at 14 days, versus 171/265 (64.5%) standard-of-care patients (estimated difference −6%, 95% CI −15 to 2%; predefined non-inferiority margin -13%). Secondary mortality and ΔSOFA outcomes narrowly favoured the control arm, without clear statistical significance.

Conclusions: In-ICU PCR for pathogens resulted in improved antibiotic stewardship. However, non-inferiority was not demonstrated for cure of pneumonia at 14 days. Further research should focus on clinical effectiveness studies to elucidate whether antibiotic stewardship gains achieved by rapid PCR can be safely and advantageously implemented.

Keywords: Hospital-acquired pneumonia (HAP), Ventilator-associated pneumonia (VAP), Molecular diagnostics, Syndromic PCR, Rapid PCR, Point-of-care, Antibiotic stewardship

Hospital-acquired and ventilator-associated pneumonias (HAP and VAP) occur in 5–40% of intensive care unit (ICU) patients, increasing morbidity and costs [1, 2]. Mortality is estimated at 10–50%, being highest in immunosuppressed patients [2–4]. Early effective antibiotics improve outcomes, but routine microbiological investigation requires 48–72 h to provide results [5]. Consequently, patients with healthcare-associated pneumonia (HAP) and ventilator-associated pneumonia (VAP) are given empirical broad-spectrum antibiotics, refined once laboratory data become available [6]. US/European consensus strategies [7] aim to minimise the development of HAP/VAP and to optimise antibiotic therapy; nevertheless, guidelines [8] continue to advocate broad-spectrum antibiotic combinations, hazarding collateral damage and selection of antibiotic resistance.

Numerous bacteria, viruses and fungi can cause HAP and VAP. Culture remains the gold-standard method of investigation despite slow turnaround and failure to identify pathogen(s) in up to 50% of cases [9]. Rapid multiplex PCR tests (also called 'syndromic' panels), seeking pathogen(s) and resistance genes, offer increased speed and sensitivity, potentially improving outcomes and antibiotic stewardship. We and many others [9–13] have demonstrated the excellent diagnostic performance of these systems in detecting key pathogens and antibiotic resistances. However, evidence of their clinical impact remains scanty, and the UK National Institute for Health and Care Excellence highlights rapid testing in HAP as a research priority. [14]

We conducted a pragmatic multi-centre RCT ('INHALE WP3' [15, 16]), investigating the utility—in respect of clinical outcomes and antibiotic stewardship—of a rapid, in-ICU syndromic PCR test (electronic supplementary table S1) for the microbiological investigation and informed targeted treatment of HAP and VAP.

Take-home message

This randomised trial provides multi-centre evidence that rapid syndromic PCR, delivered at the point-of care in the ICU improved antibiotic stewardship by 21% in absolute terms. Equivalence of clinical cure was not demonstrated and more research on clinical impact is urgently needed. A holistic approach, including behavioural intervention to optimise antibiotic prescribing, is likely needed to fully realise the potential benefits of rapid diagnostics and their role in mitigating AMR

Methods

Study design and participants

This open-label RCT recruited participants at 14 ICUs (11 adult, 3 paediatric) in 13 hospitals (12 NHS, 1 private; electronic supplementary table S3). Eligible patients were about to receive initial empiric antibiotic therapy for clinically diagnosed HAP or VAP, or about to have their antibiotic therapy changed owing to clinical deterioration of HAP or VAP, which were defined as pneumonia developing > 48 h after hospital admission or ventilation, respectively [7]. Patients, who could be ventilated or breathing spontaneously, needed to provide a lower airway specimen sufficient for routine testing, plus 200 µl for the PCR test. We excluded patients who (i) had previously participated in the trial, (ii) were participating in another interventional trial, (iii) were moribund and/or not expected to live > 48 h, or who had an existing directive to withhold life-sustaining treatment, including antibiotics. Data were collected for each patient for up to 28 days. The protocol was published previously, including amendments

necessitated by the exigencies of the COVID-19 pandemic [15].

Ethics approval was from the London-Brighton and Sussex Research Ethics Committee (19/LO/0400). Consent was deferred: adult patients or their consultees were approached for written consent or assent as soon as possible after randomisation. When incapacitated patients regained capacity, they were approached for retrospective consent directly. For children, the parents or guardians were approached for consent, and older children approached for assent. The trial was registered as ISRCTN16483855 on 5th August 2019.

Randomisation

Patients were randomly allocated (1:1) to the intervention and control groups using a centrally managed web-based system (REDCap) hosted by the Norwich Clinical Trials Unit (NCTU); randomisation was stratified by hospital, using permuted block allocation of randomly varying lengths. Assignments were concealed from all team members before randomisation; subsequently the trial was open-label at the sites.

Procedures

Patients in each group had a lower respiratory tract specimen (sputum, endotracheal aspirate, non-directed soft catheter lavage or bronchoalveolar lavage) collected before randomisation. For patients in the intervention group, part of the sample was tested, as swiftly as possible, using the FilmArray Torch Pneumonia Panel *Plus* (bioMérieux) platform (electronic supplementary table S1) [13]. This test, with a run-time of *c.* 70 min, was performed in the ICU by members of the clinical team, who had received appropriate training. Regular quality control assays were performed. The ICU care team were immediately provided with the results and a localised antibiotic prescribing algorithm [15] translating the test's results to prescribing advice. The algorithm advocated narrow-spectrum agents wherever possible. Its use was encouraged but not mandated. The remaining intervention arm sample was sent to the local microbiology laboratory for culture and susceptibility testing, performed according to national standards. [17]

For patients in the standard-of-care group a portion of each sample was frozen at < − 20 °C within 24 h, whilst the remainder underwent standard testing (as above). Patient care and treatment followed the site's standard pathways, with empirical antibiotic treatment reflecting local guidelines, generally based on international recommendations advocating broad-spectrum therapy. Batched frozen samples were shipped to one of two central laboratories and tested on the identical PCR test platform. These results were not provided to clinical teams, but were used by the Stewardship Committee.

Outcomes

The trial had co-primary outcomes of:

1. Superiority in antibiotic stewardship at 24 h post-randomisation, defined as: proportion of patients on antibacterially appropriate and proportionate antibiotic therapy within 24 h of clinical diagnosis, where 'antibacterially appropriate' was defined as receiving an antibiotic antibacterially active against the organism(s) in vitro and 'proportionate' as antibacterially appropriate and not excessively broad-spectrum for the pathogen(s) identified.
2. Non-inferiority in clinical cure of pneumonia at 14 days post-randomisation. Cure was defined as the absence of (i) death, where pneumonia was considered causative or contributory, (ii) septic shock, except when associated with a documented non-respiratory origin of infection, (iii) relapse of pneumonia (defined as an infectious pulmonary event, associated with clinical and radiological signs of HAP or VAP, or a worsening of 2 points of the baseline multiple organ dysfunction score (SOFA or PELOD-2)) or (iv) other evidence that the original pneumonia was not cured.

Secondary outcomes comprised:

(i) ICU length of stay, calculated from randomisation to discharge or death (whichever was sooner); (ii) number of ventilator-free days up to 21 days post-randomisation; (iii) death from any cause within 28 days of randomisation; incidence of septic shock within 21 days of randomisation; (iv) change in SOFA (ΔSOFA) [18] score from randomisation to 7 days post-randomisation for adults; (v) change in PELOD-2 (ΔPELOD-2) [19] score from randomisation to 7 days post-randomisation for children; (vi) change in pSOFA (ΔpSOFA, paediatric SOFA) [20] score from randomisation to 7 days post-randomisation for children; (vii) proportion of patients, at 24 and 72 h post-randomisation, on antibiotics antibacterially appropriate/inappropriate against the pathogen(s) found; (viii) proportion of patients on proportionate/disproportionate antibiotics in relation to pathogen(s) found at 72 h post-randomisation; (ix) proportion of patients on narrow-spectrum antibiotics at 24 and 72 h post-randomisation; (x) proportion of patients with specific adverse events associated with antibiotics within 21 days from randomisation; (xi) proportion of patients contracting a secondary pneumonia within 21 days from randomisation; (xii) total per-patient antibiotic usage

in WHO-recommended defined daily doses (DDDs) to 21 days post-randomisation (all conditions).

Adverse events were recorded until Day 21 and reviewed throughout by the trial committees. Due to the co-morbidities of the ICU population, events were only reported if the investigator considered them 'unusual or 'notable' for the patient. Serious adverse events (SAEs) did not require expedited reporting unless, in the opinion of the investigator, the event was related to PCR or laboratory error.

For each patient a Stewardship Committee reviewed whether treatment was antibacterially appropriate and proportionate at 24 and 72 h post-randomisation in the light of all microbiological data from culture and molecular testing, including PCR results for standard-of-care group patients. The Committee met regularly as a group and was blinded to the patient's study group and eventual outcome. Disagreements among the members were resolved by an independent adjudicator who did not attend review meetings. The Committee's terms of reference were published [15].

Sample size justification

The trial sought to recruit 552 patients over 24 months, aiming to achieve an overall power of 90% with a significance level of 5% for its two co-primary outcomes. We initially assumed a 70% clinical cure rate for ICU HAP/VAP, based on the literature and earlier work (INHALE WP2, unpublished) [21–24]. This was adjusted to 55% following the advent of COVID-19, informed by the anticipated inclusion rate of COVID-19 patients and a blinded audit of the early clinical cure rate for this subgroup. The non-inferiority limit was defined as 13%, on the basis of consensus from published trials in using this endpoint in HAP and VAP and reflecting the heterogeneity of the ICU patient population [22–25]. We estimated, based upon INHALE WP2 (unpublished) that, under standard care, 53% of patients received antibiotics that were both antibacterially appropriate and proportionate within 24 h of clinical diagnosis [26]; it was considered important to improve this by at least 20% in absolute terms (to 73%). A sample size of 552 patients (allocated 1:1, intervention: standard care) provided 91% power for the clinical non-inferiority outcome analysis and 99% power for superiority in stewardship outcome, resulting in 90% power for the co-primary analysis ($0.91 \times 0.99 = 0.9$), under the conservative assumption of no correlation between the outcomes [27]. The sample size was inflated for up to 5% attrition but not for non-compliance, as none was expected. During the trial, and following a strong recommendation from the Data Monitoring Committee, a decision was made to use standard 2-sided 95% confidence intervals for non-inferiority analyses; this resulted in a combined power of 85% for co-primary analyses under the conservative scenario of no correlation between the outcomes.

Statistical analysis

For each co-primary outcome, the effect of the intervention versus the control was estimated as a difference in proportions with a 95% confidence interval. These estimates were obtained from mixed effects binomial models with an identity link and with study site included as a random effect. For both outcomes, odds ratios were obtained using mixed effects logistic models with a random effect for site. In additional analyses, models were re-fitted including adjustments for potential confounders such as age (years), SOFA/pSOFA (continuous score), and bloodstream infection in the 7 days preceding randomisation (yes/no). A separate adjusted model included COVID-19 infection at randomisation (yes/no). For these adjusted analyses, baseline SOFA and pSOFA scores were rescaled and combined using z score transformations; missing baseline values were imputed using mean imputation [28].

Both primary outcomes were analysed for the intention-to-treat population comparing the groups as randomised, regardless of compliance. For clinical cure, a 'per-protocol' analysis was also conducted excluding intervention group patients for whom PCR test results were not obtained within 24 h of sample collection. We report analyses including cases with outcome data, without imputation of missing values. To consider the impact of missing data, sensitivity analyses were conducted using multiple imputation to complete missing values.

Similar analytic methods were used for binary secondary outcomes (mortality, septic shock, and proportions of patients receiving antibacterially appropriate, proportionate and narrow-spectrum antibiotics). Where the number of events was small, analyses did not account for site, and estimates were obtained using recommended methods [29]. For continuous clinical measures (SOFA, pSOFA and PELOD-2), groups were compared using mixed effects regression models to obtain differences in means, allowing for site as a random effect and adjusting for baseline score. A similar model was used to analyse DDDs of antibiotics, without baseline adjustment. 'Ventilator-free days' was analysed as an ordinal outcome, owing to 'zero' values for the many patients ventilated throughout. A mixed effects ordinal logistic regression included site as a random effect and estimated the treatment effect as an odds ratio. Length of ICU stay was compared between groups using a Cox competing risks survival model for death and discharge; patients alive and still in ICU at 28 days, or lost to follow-up, were censored. Death within 28 days of randomisation was also

analysed as a time-to-event outcome using a Cox model with gamma distributed shared frailty for site; those alive at 28 days or lost to follow-up were censored. For all secondary outcomes, results were reviewed before and after adjustment for the same set of baseline factors as for the primary outcomes. All analyses compared groups as randomised, using available data. There was no allowance for multiplicity in analyses of secondary outcomes.

Data were analysed using STATA version 17. Analysis followed a pre-specified statistical analysis plan approved by INHALE's Data Monitoring Committee; this was made available before analyses began (https://norwichctu.uea.ac.uk/inhale-publications/).

For further details on methodology, please see the supplementary methods section.

Results

Between 5th July 2019 and 18th August 2021, 554 eligible patients were randomised to the intervention ($n=277$) and standard-of-care ($n=277$) groups, achieving the recruitment target. Recruitment was paused between 16th March 2020 and 9th July 2020 owing to the COVID-19 pandemic. Subsequently, both COVID-19 and non-COVID-19 patients were accepted. Nine randomised patients retrospectively withdrew consent and were excluded from all analyses, leaving data for 453 adults and 92 children (Fig. 1, Table 1, electronic supplementary tables S3, S4, S5 and S6). Four patients were randomised but subsequently found ineligible (based on pre-randomisation information) and excluded. Two intervention group patients lacked PCR results and are omitted from 'per protocol' analyses; 12 were lost owing to transfer to other hospitals within 14 days of randomisation and 6 withdrew from antibacterially appropriate follow-up. Primary outcomes were available for 97% of eligible and consenting patients ($n=531$ for stewardship, $n=533$ for clinical cure).

Patients were predominantly male (68.4%); adults had a median age of 61 years (interquartile range (IQR) 49–71), children had a median age of 7.5 months (IQR 2–33.5) (Table 1, electronic supplementary table S4) Baseline characteristics were well balanced between the groups; 183 eligible patients (33.6%) had COVID-19 at randomisation, all recruited after the study re-opened on 9th July 2020. Baseline rates of multi-drug resistant organisms were low. Syndromic PCR results were available in a median time of 1.5 h (IQR 1.4–1.8), compared with a median of 73.7 h (IQR 66.5–116.7) for standard culture results. Comparable pneumonia pathogens were identified in the two study groups (electronic supplementary tables S7, S8 and S22).

Co-primary outcome

Intention-to-treat (ITT) analysis for the co-primary superiority (stewardship) outcome showed that 205/268 (76.5%) intervention group patients were receiving antibacterially appropriate and proportionate antibiotics by 24 h after randomisation, as adjudged by the Stewardship Committee, versus 147/263 (55.9%) in the control group (estimated difference after accounting for site 21%, 95% Confidence interval (CI) 13–28%, [odds ratio (OR) 2.57 95% CI 1.77–3.73]) (Table 2). Sensitivity analyses, and analyses adjusted for potential confounders, yielded similar results (data not shown).

In respect of the clinical co-primary outcome: 152 of 268 (56.7%) intervention group patients had clinical cure of pneumonia at 14 days versus 171/265 (64.5%) control patients. The estimated difference, after accounting for site, was −6%, with 95% confidence limits of −15% to 2%. These values overlap the non-inferiority margin of 13%, meaning that non-inferiority was not established. Results were similar in a per-protocol analysis excluding 2 intervention-group patients lacking PCR results (Table 3). Adjusting for age, baseline SOFA/pSOFA, COVID status and bloodstream infection in the 7 days preceding randomisation slightly reduced the estimated difference and confidence interval (difference −5% (95% CI −12% to 3%)), with the lower limit now falling just within the non-inferiority region; however, other adjusted analyses and sensitivity analyses left the lower bound of the confidence interval just below the non-inferiority margin (electronic supplementary table S9).

Secondary outcomes

Analyses of secondary outcomes supported the primary stewardship results, with stewardship improvements consistently apparent for the intervention group (Table 2). Thus, more intervention group patients had antibacterially appropriate and proportionate antibiotics at 72 h and more received antibacterially appropriate antibiotics (irrespective of proportionality) at 24 h and 72 h, with differences relative to the control group being significantly greater than zero (Table 2). Receipt of narrow-spectrum antibiotics was infrequent (91 of 539 patients, 16.9% at 24 h), with no evidence of significant differences between groups at 24 h or 72 h (Table 2). Antibiotic consumption was measured up to 21 days post-randomisation and found to have a mean of 1.2 (standard deviation (SD) 1.1) DDDs/ICU-day in the intervention arm versus 1.3 (SD 1.3) in the control group. Electronic supplementary figure S1 and electronic supplementary table S10 show total consumption for selected antibiotics. Although overall differences in total consumption over 21 days were small, control group patients generally received more broad-spectrum antibiotics, principally

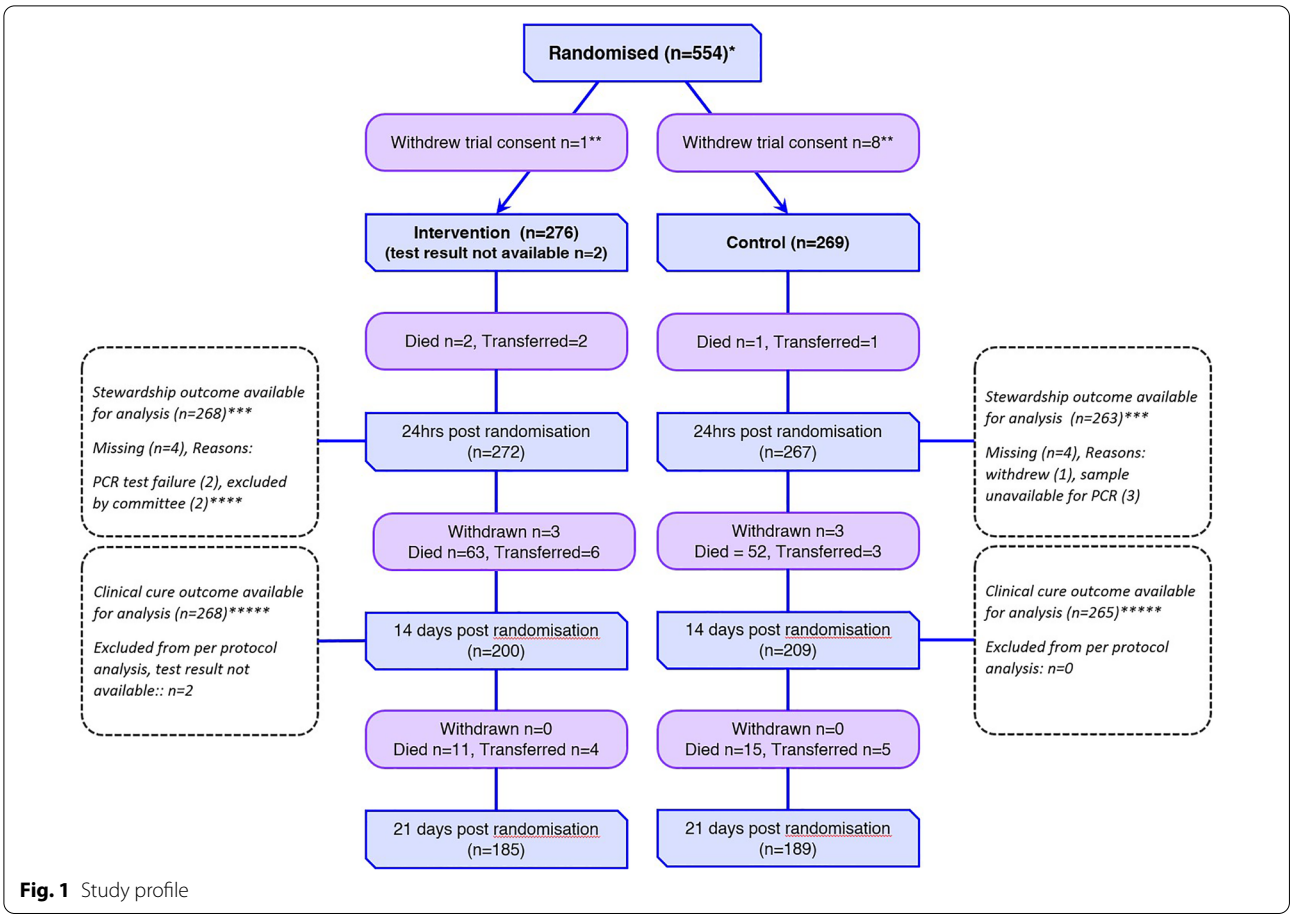

Fig. 1 Study profile

aminoglycosides, carbapenems and piperacillin–tazobactam, whereas intervention group patients received more narrow-spectrum drugs.

Clinical secondary outcomes are summarised in Table 3: 28-day mortality was 31.3% in the intervention group (85/272 patients) and 28.2% in the control group, (75/266). The estimated difference after accounting for site was 5% (95% confidence interval (−1 to −11%). A Kaplan–Meier plot shows a raised risk of death for the intervention group, but this was not significant in a Cox regression analysis accounting for site (Fig. 2, Table 3, electronic supplementary tables S11 and S12). There was no evidence of differences between groups for ICU length of stay or ventilator-free days.

Progression of organ dysfunction was measured in both adults and children (Table 4, electronic supplementary tables S13-S15 for additional adjusted analyses). For the adult population, baseline SOFA at randomisation was 6.8 (SD 3.0) in the intervention group, versus 7.1 (SD 3.0) in the control group (Table 1). These scores then reduced over 7 and 14 days, indicating clinical improvement, with marginally larger decreases for the control group compared with the intervention. For the paediatric population, mean baseline pSOFA at randomisation was 4.7 (SD 2.3) for the intervention group and 4.9 (SD 1.9) for the control group (Table 1). These values also decreased over time in both groups, with a slightly larger decrease in the control group by 14 days. Differences between groups were small and unlikely to be clinically meaningful.

In analyses of secondary outcomes considered to be antibiotic-associated adverse events (Table 3), there was no evidence of a difference between the groups for septic shock, severe antibiotic hypersensitivity, secondary pneumonia, nor—based on very few cases—for *Clostridium difficil*e superinfection. Antibiotic-associated diarrhoea was more frequent in the intervention group, occurring in 26/263 (9.9%) patients, versus 14/257 (5.5%) in the control group (estimated difference after accounting for site 4% (95% CI 0.1–9%) (electronic supplementary table S16 provides a list of antibiotics administered to those who experienced diarrhoea). For other adverse events there were no trends either in number (7 in each arm) nor nature (electronic supplementary table S17). No serious trial-related events were reported.

Table 1 Baseline patient characteristics by randomised group

	Intervention ($n=276$)		Standard of care ($n=269$)
Demographics			
Male	184 (66.7%)	189 (70.3%)	
Adults (18+ years)	228 (82.6%)	225 (83.6%)	
Age in adults (years)	58.2 (16.2) [60 (47–71)]	59.4 (15.0) [61 (52–70)]	
Children (< 18 years)	48 (17.4%)	44 (16.4%)	
Children (< 2 years)	34 (12.3%)	31 (11.5%)	
Age in children (months)	31.6 (50.5) [5.5 (1.5–29)]	33.2 (53.0) [8.5 (2.5–38)]	
Ethnicity			
White British	147 (53.3%)		147 (54.7%)
White other	29 (10.5%)		35 (13.0%)
Indian, Pakistani or Bangladeshi	17 (6.2%)		15 (5.6%)
Asian other	19 (6.9%)		13 (4.8%)
Black Caribbean	1 (0.4%)		3 (1.1%)
Black African	5 (1.8%)		2 (0.7%)
Black other	12 (4.4%)		10 (3.7%)
Mixed race	4 (1.4%)		10 (3.7%)
Any other	6 (2.2%)		14 (5.2%)
Not stated	36 (13.0%)		20 (7.4%)
Co-morbidities (yes/no for each)			
SARS-CoV-2 infection at randomisation[a]	93 (33.7%)		90 (33.5%)
Missing/unknown SARS-CoV-2 infection at randomisation[a]	25 (9.1%)		24 (8.9%)
Bloodstream infection in 7 days prior to randomisation	7 (2.5%)		18 (6.7%)
Missing	1 (0.4%)		1 (0.4%)
Abdominal	30 (10.9%)		26 (9.7%)
Cardiovascular	126 (45.7%)		128 (47.6%)
Cancer—haematological	11 (4.0%)		12 (4.5%)
Cancer—solid tumour	35 (12.7%)		28 (10.4%)
Chronic kidney disease/renal failure	15 (5.4%)		15 (5.6%)
Chronic lung disease	58 (21.0%)		52 (19.3%)
Chronic liver disease/ cirrhosis	8 (2.9%)		17 (6.3%)
Congenital cardiac malformation (excluding PDA, secundum ASD)	17 (6.2%)		20 (7.4%)
Congenital, other	12 (4.3%)		11 (4.1%)
COPD	29 (10.5%)		22 (8.2%)
Diabetes	55 (19.9%)		56 (20.8%)
Immunocompromised	14 (5.1%)		13 (4.8%)
Mental Health	26 (9.4%)		29 (10.8%)
Neurological	21 (7.6%)		19 (7.1%)
Post-operative	63 (22.8%)		57 (21.2%)
Rheumatological	19 (6.9%)		21 (7.8%)
Known colonisation by MRSA	1 (0.4%)		2 (0.7%)
Known colonisation by ESBL producer	1 (0.4%)		2 (0.7%)
Known colonisation by carbapenemase producer	1 (0.4%)		2 (0.7%)
ICU admission type			
Medical	194 (70.3%)		190 (70.6%)
Surgical	59 (21.4%)		52 (19.3%)
Trauma	16 (5.8%)		20 (7.4%)
Other	7 (2.5%)		7 (2.6%)
ICU admission source			

Table 1 (continued)

	Intervention ($n=276$)	Standard of care ($n=269$)
Elective admission	18 (6.5%)	18 (6.7%)
From emergency department	86 (31.2%)	77 (28.6%)
From elsewhere in hospital	112 (40.6%)	111 (41.3%)
From another hospital	60 (21.7%)	63 (23.4%)
Type of pneumonia		
HAP (all)	84 (30.4%)	87 (32.4%)
HAP (invasive ventilation at randomisation)	50 (18.1%)	53 (19.8%)
VAP	191 (69.2%)	182 (67.7%)
Data missing	1 (0.4%)	0
Ventilation status at randomisation		
Not ventilated	31 (11.2%)	33 (12.3%)
Ventilated: non-invasive	10 (3.6%)	7 (2.6%)
Ventilated: invasive	234 (84.8%)	228 (84.8%)
Data missing	1 (0.4%)	1 (0.4%)
LRT sample type		
Endotracheal tube aspirate	185 (67.0%)	183 (68.0%)
Bronchoalveolar lavage	43 (15.6%)	36 (13.4%)
Non-directed bronchoalveolar lavage	4 (1.5%)	8 (3.0%)
Sputum	37 (13.4%)	33 (12.3%)
Other	7 (2.5%)	9 (3.4%)
Received antibiotics for any indication in 7 days prior to randomisation	255 (92.4%)	245 (91.2%)
APACHE II score at ICU admission (adults) Range: 0 (good)–55 (poor)	24.6 (9.3) [25 (16.5–31)] ($n=204$)	23.5 (7.9) [24 (17–29)] ($n=199$)
SOFA score in adults at randomisation ($n=454$)[b]	6.8 (3.0) ($n=228$)	7.1 (3.0) ($n=226$)
PIM3 at ICU admission (children) (probability of death: 0–1.0)	0.11 (0.19) [0.05 (0.03–0.11)] ($n=48$)	0.11 (0.13) [0.06 (0.02–0.15)] ($n=43$)
pSOFA score in children at randomisation ($n=91$)[b]	4.7 (2.3) ($n=48$)	4.9 (1.9) ($n=43$)
PELOD-2 score in children at randomisation ($n=91$)[b]	5.1 (1.9) ($n=48$)	6.0 (2.3) ($n=43$)

Data are *n* (%), mean (SD) or [median (IQR)]

[a] The method for determining SARS-CoV-2 status depended on time of randomisation. Routine ICU SARS-CoV-2 screening data were collected for all patients after 2 July 2020. Prior to 16 March 2020, the study did not formally recruit COVID-19 patients, but we recognised that there may have been unknown cases of SARS-CoV-2 in early 2020. Accordingly, available frozen samples were retrospectively tested for SARS-CoV-2 by PCR but did not recover any positives among 55 samples from patients recruited between 1 January and 16 March 2020. We have assumed all those recruited prior to 1 January 2020 were SARS-CoV-2 negative. Those recruited between 1 January 2020 and 16 March 2020 where no sample was available for testing have been treated as unknowns

[b] Score on Day 1 for those patients randomised on Day 1, and Day 2 for those patients randomised on Day 2. Missing values imputed with mean values

Post hoc analyses

Post hoc investigations were conducted to better understand the reasons for the failure to demonstrate non-inferiority for clinical cure, and for the parallel observations that mortality and evolution of sequential organ failure assessment (SOFA) scores tended to favour the control group. We found that, among patients in whom a pathogen was identified and who were receiving antibacterially appropriate and proportionate antibiotic treatment at 24 h, the cure rate was 55.5% in the intervention group, versus 67.8% in the control group, a significant difference (unadjusted difference −12.3% (95% CI −22.5 to −2.1%) (electronic supplementary table S18). On the other hand, cure rates amongst patients for whom stewardship aims were *not* achieved were much more similar between the trial arms, with no statistical evidence of a difference. We descriptively reviewed algorithm adherence and its relationship to clinical cure by randomisation group (electronic supplementary table S19). Treatment was considered adherent only if it exactly matched the algorithm recommendation for any pathogen(s) found by both PCR and culture. Summaries are shown for both

Table 2 Antibiotic stewardship-related primary and secondary outcomes

	Intervention	Standard of care	Treatment effect estimates (intervention vs standard of care)[a]	
	n/N (%)	*n/N* (%)	Difference in proportions (95% CI)	Odds ratio (95% CI)
Antibacterially appropriate and proportionate antibiotics at 24 h (*n* = 531)				
Total population	205/268 (76.5%)	147/263 (55.9%)	0.21 (0.13 to 0.28)	2.57 (1.77 to 3.73)
Adults only	173/223 (77.6%)	123/219 (56.2%)	0.21 (0.13 to 0.30)	2.70 (1.79 to 4.08)
Antibacterially appropriate antibiotics at 24 h (*n* = 531)				
	245/268 (91.4%)	204/263 (77.6%)	0.13 (0.07 to 0.20)	3.12 (1.86 to 5.25)
Antibacterially appropriate and proportionate antibiotics at 72 h (*n* = 516)				
	185/252 (73.4%)	150/255 (58.8%)	0.15 (0.07 to 0.23)	1.95 (1.34 to 2.85)
Antibacterially appropriate antibiotics at 72 h (*n* = 516)				
	230/252 (91.3%)	208/255 (81.6%)	0.10 (0.04 to 0.16)	2.36 (1.38 to 4.06)
Patients on narrow-spectrum antibiotics at 24 h (*n* = 539)				
	47/272 (17.3%)	44/267 (16.5%)	0.005 (−0.06 to 0.07)	1.06 (0.67 to 1.68)
Patients on narrow-spectrum antibiotics at 72 h (*n* = 516)				
	74/257 (28.8%)	61/259 (23.6%)	0.05 (−0.02 to 0.13)	1.32 (0.88 to 1.97)
	Mean (SD) [Median (IQR)]			Difference in means (95% CI)
DDD of antibiotics administered in ICU, up to 21 days (*n* = 526)				
Total DDD	14.3 (15.8) [8.5 (3.5 to 18.4)] *n* = 264		15.1 (17.3) [7.9 (4.2 to 20.4)] *n* = 262	–
DDD/ day in ICU	1.2 (1.1) [1.0 (0.5 to 1.7)] *n* = 264		1.3 (1.3) [1.0 (0.5 to 1.6)] *n* = 262	−0.08 (−0.26 to 0.11)

SD standard deviation, *CI* confidence interval, *IQR* interquartile range

[a] ITT comparison based on mixed effects model with a random effect for study site

trial arms although, for the control group, any correspondence with the algorithm was purely coincidental. Compliance with the algorithm in the intervention group was low, at only 30.5% (58/190) among those with at least one potential pathogen identified. These had a higher rate of cure (65.5%, 36/55) than intervention group patients for whom the algorithm was not followed (58.0%, 76/131) or in whom no pathogen was identified (48.8%, 40/82). Patients with treatment that was (coincidentally) consistent with the algorithm in the control group had a higher rate of cure (93.5%, 29/31) than those in the equivalent intervention group. For further post hoc analyses see supplementary results (electronic supplementary figure S2, electronic supplementary tables S20, S21).

Discussion

INHALE WP3 was a pragmatic trial, recruiting any critically ill adult or child with clinically suspected or confirmed HAP or VAP about to start or change antibiotics. These criteria were chosen to reflect "real-life" medical practice and to provide information for a broad population. Delays in the "time-to-antibiotic decision" were minimised by placing the diagnostic in the ICU and providing a prescribing algorithm, tailoring treatment to the pathogen(s) and antibiotic resistance gene(s) found. Consequently, PCR results were typically available in under 2 h vs. a median of 73.7 h for routine culture results. Delays in delivery of routine culture results were reflective of a variety of factors including pandemic related disruption, use of off-site laboratories and non-7 day working patterns. In the intervention arm, 70.3% of participants (electronic supplementary table S7) had a pathogen identified by PCR, culture, or both, comparing favourably the reported performance of culture alone, ranging from 30 to 50% [9].

Use of the syndromic multiplex PCR led to a 21% absolute improvement in antibiotic stewardship (95% CI 13–29%) defined as the proportion of HAP and VAP patients receiving antibacterially appropriate and proportionate therapy 24 h post-diagnosis. This advantage

Table 3 Primary and secondary clinical outcomes

	Intervention	Standard care	Treatment effect estimates (intervention vs. standard care)[a]	
	n/N (%)	*n/N*(%)	Difference in proportions (95% CI)	Odds ratio/hazard ratio (95% CI)
Clinical cure at 14 days				
'Intention to treat' analysis	152/268 (56.7%)	171/265 (64.5%)	−0.06 (−0.15 to 0.02)	OR 0.68 (0.47 to 0.98)
'Per protocol' analysis	150/266 (56.4%)	171/265 (64.7%)	−0.06 (−0.15 to 0.02)	OR 0.68 (0.47 to 0.98)
'Intention to treat' analysis, adults only	117/224 (52.2%)	136/222 (61.3%)	−0.09 (−0.18 to 0.001)	0.68 (0.46 to 1.00)
All-cause mortality at 28 days (*n* = 545)				
	85/272 (31.3%)	75/266 (28.2%)	0.05 (−0.01 to 0.11)	HR 1.18 (0.87 to 1.61)
Adverse event: septic shock within 21 days of randomisation (*n* = 519)				
	37/262 (14.1%)	31/257 (12.1%)	0.03 (−0.01 to 0.07)	1.23 (0.72, 2.10)
Adverse event: antibiotic-induced diarrhoea within 21 days of randomisation (*n* = 520)				
	26/263 (9.9%)	14/257 (5.5%)	0.04 (0.001 to 0.09)[b]	1.95 (0.97 to 3.93)
Adverse event: *Clostridium difficile* infection within 21 days of randomisation (*n* = 521)				
	3/263 (1.1%)	5/258 (1.9%)	−0.01 (−0.03 to 0.02)[b]	–
Adverse event: severe antibiotic hypersensitivity within 28 days of randomisation (*n* = 520)				
	1/263(0.4%)	2/257 (0.8%)	0.00 (−0.02 to 0.01)[b]	–
Adverse event: secondary pneumonia within 21 days of randomisation (*n* = 519)				
	25/263 (9.5%)	31/256 (12.1%)	−0.03 (−0.08 to 0.03)[b]	0.76 (0.43 to 1.23)
Adverse event: other (*n* = 538)[c]				
	7/272 (2.6%)	7/266 (2.6%)	0.00 (−0.03 to 0.03)[b]	–
ICU length of stay, days (up to 28 days)				
	Median (IQR)			
All patients (n = 539)	11 (6 to 25) (*n* = 274)	13 (6 to 26) (*n* = 265)	–	HR 0.95 (0.82 to 1.10)
Patients surviving to/discharged within 28 days (*n* = 393)	14 (7 to 28) (*n* = 196)	14 (7 to 28) (*n* = 197)	–	–
Patients not surviving to day 28	7 (4 to 12) (*n* = 78)	10 (5 to 15.5) (*n* = 68)	–	–
Ventilator- free days (up to day 21)				
	Median (IQR)			
All patients (*n* = 517)	2 (0 to 16) (*n* = 261)	2 (0 to 16.5) (*n* = 265)	–	OR 0.98 (0.72 to 1.35)
Patients surviving to day 21 (*n* = 371)	11 (1 to 18) (*n* = 184)	9 (1 to 18) (*n* = 187)	–	OR 1.10 (0.77 to 1.58)

SD standard deviation, *CI* confidence interval, *IQR* interquartile range, *OR* odds ratio, *HR* hazard ratio or sub hazard ratio from competing risks model for the time until discharge (length of stay) analysis, – not calculated

[a] ITT comparison based on mixed effects model with a random effect for study site, unless specified otherwise

[b] Due to small numbers, analyses did not account for site and confidence intervals were obtained using methods proposed by Agresti and Caffo [27]

[c] For a detailed listing see electronic supplementary table S17

persisted at 72 h. This manifested as more tailored antibiotic therapy, rather than substantial changes in escalations or de-escalations (electronic supplementary table S22, data not shown). PCR was run retrospectively for control arm patients, so that stewardship assessment was based on an identical set of results: the proportion of control arm patients with a pathogen identified by on-site routine microbiology was 47.2%; this rose to 76.6% when the retrospective PCR was run, mirroring the intervention arm (data not shown). These stewardship gains compare favourably with those from other interventions. Nonetheless, INHALE WP3 failed to confirm clinical non-inferiority at 14 days, with a 6% lower cure rate for the intervention group, and with the lower confidence limit falling below the −13% non-inferiority margin. Secondary clinical outcomes—mortality and evolution of the SOFA score—also tended to favour the control group but differences were small and a Cox regression analysis did not show an increased risk of death in the intervention group. Given these borderline results, uncertainty

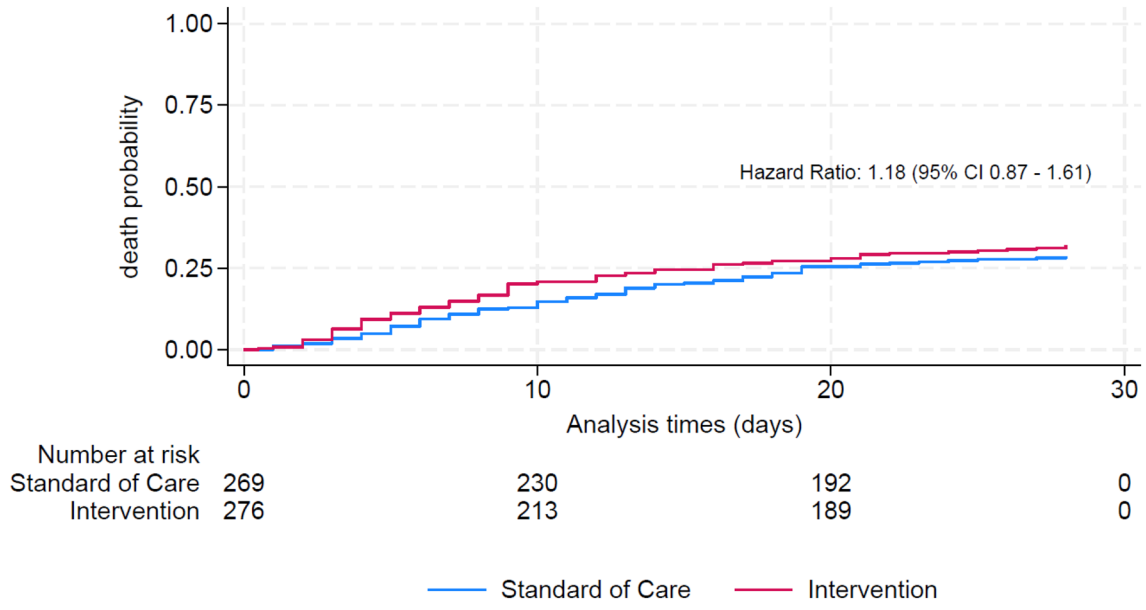

Fig. 2 Kaplan–Meier plot for mortality

remains whether we observed a small but meaningful effect in favour of the control group or just 'noise', which commonly affects ICU trials owing to population heterogeneity [30]. Health economic analyses found a cost saving of £8214 per patient in the intervention arm, despite the cost of the test (INHALE, unpublished).

Many previous evaluators assert, based upon laboratory results, that rapid diagnostics *might* improve antibiotic prescribing. Translating potential gains to clinical practice is less certain. The MultiCov study, applying syndromic PCR and procalcitonin levels in severe COVID-19 patients, failed to show an impact on antibiotic use or clinical outcomes [31]. Other studies are more positive [32–34]: (i) the FLAGSHIP-II trial, testing a similar diagnostic test (Curetis Unyvero), recorded shorter inappropriate treatment in the intervention group [32], and (ii) a single-centre RCT using the same PCR as here found that 80% of intervention patients received results-directed antibiotic therapy vs. 29% of control patients receiving culture-guided therapy [33]. However, both these latter trials incorporated in-person or telephone 'nudge' advice from a microbiologist for intervention-group patients. Here, we achieved improved stewardship without any 'nudge'; however, considerable room for improvement remained, as only 30.5% of intervention-arm patients with a pathogen found received the antibiotics advocated in the treatment algorithm. We also noted many (47/255, 18.4%) control group patients still on *antibacterially inappropriate* antibiotics at 72 h. Failure of culture-based methods to detect pathogens due to high levels of antibiotic usage may have been a contributory factor, given the large proportion (c. 90%) of patients already on therapy. These finding demonstrate that successful implementation of point-of-care PCR will require additional behavioural strategies to enhance compliance and optimised usage [35].

The failure to meet the pre-set non-inferiority margin for clinical cure was unexpected as was the finding, from exploratory analyses, that the patients driving this result were those *from whom a pathogen had been detected and who had received antibiotics deemed 'antibacterially appropriate and proportionate'* (electronic supplementary table S18). Several possible explanations exist. First, this result remains within the bounds of chance variation. Secondly, there is the issue of defining cure in pneumonia. We used 'clinical cure', as the EMA standard in antibiotic trials for pneumonia [21, 36], and provided sites with interpretive guidance, but note general issues with this outcome, such as patients failing to recover for other reasons besides continuing infection. Furthermore, the local clinicians assessing cure knew the patient's randomisation group, creating a potential for bias. The best argument against this having confounded analysis is that objective measures—mortality and evolution of organ dysfunction—tracked with it. Thirdly, we considered whether our algorithm's recommendations prompted inferior treatment. This seems unlikely: cure was more frequent in those who received treatment consistent with the algorithm in either arm compared to those who did not, although a difference between arms in favour of the

Table 4 Progression of organ dysfunction in adult and paediatric study populations

	Intervention mean (SD)	Standard of care Mean (SD)	Adjusted difference in means (95% CI)[a]
SOFA score in adults (*n* = 454)[b]			
Score at Day 7[c]	5.9 (3.8) (*n* = 227)	5.6 (3.8) (*n* = 225)	–
Score at Day 14	5.4 (4.3) (*n* = 227)	5.4 (4.0) (*n* = 225)	–
ΔSOFA (Day 7—randomisation)[d]	− 0.9 (3.1) (*n* = 227)	− 1.6 (3.3) (*n* = 225)	0.6 (0.004 to 1.1)
ΔSOFA (Day 14—randomisation)[d]	− 1.4 (3.8) (*n* = 227)	− 1.7 (3.9) (*n* = 225)	0.2 (− 0.5 to 0.9)
pSOFA score in children (*n* = 91)			
Score at day 7[c]	2.2 (2.5) (*n* = 48)	2.4 (3.2) (*n* = 43)	–
Score at Day 14	2.0 (3.1) (*n* = 48)	1.0 (1.8) (*n* = 43)	–
ΔpSOFA (Day 7—randomisation)[d]	− 2.4 (3.0) (*n* = 48)	− 2.4 (2.8) (*n* = 43)	0.1 (− 1.2 to 1.0)
ΔpSOFA (Day 14—randomisation)[d]	− 2.7 (3.6) (*n* = 48)	− 3.9 (2.3) (*n* = 43)	1.0 (− 0.0002 to 2.0)
PELOD-2 score in children (*n* = 91)			
Score at Day 7[c]	2.6 (2.7) (*n* = 48)	2.7 (3.1) (*n* = 43)	–
Score at Day 14	1.9 (2.9) (*n* = 48)	1.6 (2.6) (*n* = 43)	–
ΔPELOD-2 (Day 7—randomisation)[d]	− 2.5 (2.7) (*n* = 48)	− 3.4 (2.7) (*n* = 43)	0.5 (− 0.6 to 1.6)
ΔPELOD-2 (Day 14—randomisation)[d]	− 3.2 (3.4) (*n* = 48)	− 4.4 (3.1) (*n* = 43)	0.4 (− 0.7 to 1.6)

SD standard deviation, *CI* confidence interval, *IQR* interquartile range

[a] ITT comparison based on model adjusted for score at randomisation and with random effect for site

[b] Includes one patient aged 17 on admission

[c] Missing GCS values assumed to be 'normal'

[d] Missing baseline score imputed with mean

control was still noted. Fourth, electronic supplementary figure S2 suggests that poorer clinical outcomes in the intervention group concentrated at particular sites; however, patient numbers per site were insufficient for robust comparison, adjusted analyses also account for site as a potential confounder. The diversity of empirical therapy at different sites adds complexity (electronic supplementary tables S2 and S22) but is equalised between arms by randomisation. Fifth, there are the effects of COVID-19: cure rates were lower for COVID-19 patients (explaining a lower overall cure rate than typical of HAP/VAP studies) and differences in cure rates between groups were more pronounced for COVID-19 patients (electronic supplementary table S21). Last, there is the disturbing possibility that HAP and VAP are not, ab initio, infections caused by the few species sought by culture or multiplex PCR. Rather, the early stages of HAP/VAP may entail aspiration events, with mixed oral anaerobes, or dysbiosis of a putative lung flora, with the detected 'pathogen(s)' only subsequently gaining ascendency [37]. To our knowledge, there is no clear causal link between many organisms commonly associated with HAP and VAP and the clinical findings of purulent sputum, deteriorating gas exchange, and inflammation. In short, clinical failure may reflect additional organisms and/or inflammatory processes, undetected by classical or molecular microbiology, that are important drivers of pneumonia. Metagenomic techniques may provide insight into this possibility [38, 39]. If so, early broad antibiotic therapy may be beneficial, just as it is universally accepted for the mixed flora typical of intra-abdominal sepsis. Broader spectrum therapy may better protect the individual, short term, but at the risk of driving population resistance in the longer term. Results indicated a greater usage of broad-spectrum carbapenems and piperacillin-tazobactam in the control group to Day 21 (electronic

supplementary figure S1, electronic supplementary table S10).

A limitation is that INHALE was conducted solely in England, which has a low prevalence of antibiotic-resistance. Consequently, PCR-panel tests for antibiotic resistance genes were of infrequent value. A second limitation is that the treatment algorithm provided recommendations, not mandated regimens. Compliance was consequently low, possibly impacting outcomes. Thirdly, COVID-19 represents a potential confounder: INHALE began by recruiting 'typical' ICU patients, who developed HAP/VAP after hospitalisation for reasons unconnected to infection but, under the circumstances of 2020/21, recruited 183 patients hospitalised primarily owing to COVID-19. Since these COVID-19 patients were distributed evenly between the trial groups, this should not have distorted the primary comparisons. Notably, (i) COVID patients had worse outcomes than other groups, suppressing cure rates in both groups, and (ii) data suggest that particular bacteria, notably *Klebsiella* spp. are unusually prevalent as secondary pathogens in severe COVID patients [40].

Conclusions

INHALE WP3's results were encouraging in respect of the diagnostic's impact on antibiotic stewardship; ICU deployment maximised the speed advantage over microbiological culture, prompting enthusiasm among ICU staff [41]. Given this improved stewardship, the failure to demonstrate non-inferiority of clinical cure is puzzling and worrying, especially as post-hoc analyses demonstrated that worse cure outcomes were associated with individuals receiving 'optimal' treatment according to current antibiotic stewardship 'best-practise'.

We recommend that use of syndromic PCR to narrow antibiotic therapy should be cautious. We do not advise modification of current prescribing strategies until further data are available. Further fundamental research is needed to better understand the microbiological progression of HAP and VAP and the implications of this study for clinical practice. Use to swiftly detect resistance genes may be beneficial in settings where these are prevalent; UK prevalence is too low to properly assess this aspect.

Supplementary Information
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Author contributions
VG, VE, DML and JOG conceived the study and obtained the funding with input from DB, JB, RH, DT and AMS. VE, DML, JH, JB, DB, VG, AMS, CR, DT, MP, AMS and SSt designed the study. JH managed site set-up and the trial with

assistance from CR, VE and ZD. AC developed the study database. DB, SSi, RP, MP, NP, JK, DM, IDW, VP, HK and ET recruited patients and collected data. SSt, CR, ZD, VE and JH curated the data. JB developed the statistical analysis plan and led analysis performed by SSt. MD, BC, PR, DML and RS reviewed antibiotic stewardship outcomes. SSt, JB, VE and CR had access to all the data. VG, VE, DML, JB, SSt, DB and JH interpreted the data. VG, VE, DML, JB, JH and SSt drafted the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. VG, VE and DML act as guarantors.

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Data availability
The data dictionary and deidentified patient data analysed and presented in this study are available from NCTU following publication, on reasonable request and subject to appropriate data sharing agreements. The statistical analysis plan is publicly available at https://norwichctu.uea.ac.uk/inhale/."

Declarations

Conflicts of interest
DB reports payments for educational sessions from bioMérieux and Gilead and consultancy fees from Paion. VE reports consultancy and speaker fees from bioMérieux, personal fees from Alchemab Therapeutics, and in-kind contributions from Inflammatix Inc. JOG has reports personal fees and/or in-kind contributions and/or research funding from Oxford Nanopore Technologies (ONT), Simcere, Becton–Dickinson and Heraeus Medical. He is now an employee of ONT and holds shares in the company. VG reports speaker fees from bioMérieux and consultancy fees from Gilead, MSD, Pfizer and Shionogi. JH reports consultancy fees from bioMérieux. HK reports speaker fees from bioMérieux. DML reports personal fees from Adjutec, AstraZeneca, bioMérieux, Centauri, GenPax, GSK, Hikma, Merck/MSD, Nordic, Paion, Pfizer, Shionogi, Sumitovant, Summit, Thermofisher, Wockhardt and Zambon, He also reports shareholdings from GenPax, GSK, Merck, Oxford Nanopore and PerkinElmer/Revvity, comprising less than 10% of portfolio value. He also has nominated holdings in Arecor, Celadon Pharmaceuticals, Destiny Pharma, Eluceda Ltd., Genedrive, Poolbeg, Optibiotix, Probiotix Health, SkinBiotherapeutics, Trellus and Verici Dx (all of which have research/products pertinent to medical and diagnostic innovation) through Enterprise Investment Schemes but has no authority to trade these shares directly. All are outside the submitted work. All other authors declare no competing interests.

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