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Original research article

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Original research

Airway pathogens detected in stable and exacerbated COPD in patients in Asia-Pacific

Laura Taddei[1*], Lucio Malvisi[1*], David S. Hui[2], Ludovic Malvaux[3], Ronnie Z. Samoro[4], Sang Haak Lee[5], Yiu Cheong Yeung[6], Yu-Chih Liu[7], Ashwani Kumar Arora[1]

1. GSK, Siena, Italy
2. Department of Medicine and Therapeutics, The Chinese University of Hong Kong, and Stanley Ho Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China
3. GSK, Wavre, Belgium
4. Healthlink (Iloilo) Inc, Philippines
5. Division of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, Eunpyeong St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
6. Department of Medicine and Geriatrics, Princess Margaret Hospital, Hong Kong SAR, China
7. Department of Thoracic, Critical Care and Sleep Medicine, Chang Gung Memorial Hospital, Keelung, Chang Gung University, Taiwan

*These authors contributed equally

Corresponding author: Laura Taddei, via Fiorentina 1, Siena, Italy; +39 3451087503; laura.x.taddei@gsk.com

Summary

The presence of *Haemophilus influenzae, Moraxella catarrhalis* or human rhinovirus in sputum samples significantly increased the odds of an exacerbation, as opposed to being in stable state, in chronic obstructive pulmonary disease (COPD).

Clinical Trial Registration: www.clinicaltrials.gov NCT03151395

ABSTRACT

Background

The burden of COPD in the Asia-Pacific region is projected to increase. Data from other regions show bacterial and viral infections can trigger acute exacerbations of COPD (AECOPD).

Methods

This 1-year, prospective, epidemiological study (NCT03151395) of patients with moderate to very severe COPD in Hong Kong, the Philippines, South Korea and Taiwan assessed the prevalence in sputum samples (by culture and PCR) of bacterial and viral pathogens during stable COPD and AECOPD. The odds of experiencing an exacerbation was evaluated for pathogen presence, acquisition and apparition. Health-related quality of life (HRQOL) was assessed.

Results

197 patients provided 983 sputum samples; 226 during exacerbation episodes. The mean yearly AECOPD incidence rate was 1.27 per patient. The most prevalent bacteria by PCR at exacerbation were *Haemophilus influenzae* (Hi) and *Moraxella catarrhalis* (Mcat); Mcat prevalence was higher at exacerbation than at stable-state. Virus prevalence was low, other than for human rhinovirus (HRV: 8.1%, stable-state; 16.6%, exacerbation). The odds ratio for an exacerbation (vs. stable state) was statistically significant for presence, acquisition and apparition of Hi (2.20 [95% CI: 1.26–3.89]; 2.43 [1.11–5.35]; 2.32 [1.20–4.46], respectively), Mcat (2.24 [1.30–3.88]; 5.47 [2.16–13.86]; 3.45 [1.71–6.98], respectively) and HRV (2.12 [1.15–3.91]; 2.22 [1.09–4.54]; 2.09 [1.11–3.91], respectively). HRQOL deteriorated according to number of exacerbations experienced.

Conclusion

In patients with COPD in the Asia-Pacific region, the presence of Hi, Mcat or HRV in sputum samples significantly increased the odds of an exacerbation, providing further evidence of potential roles in triggering AECOPD.

Keywords: Asia-Pacific; AECOPD; bacteria; burden of disease; COPD; Hong Kong; Korea; prevalence; quality of life; Philippines; Taiwan; virus

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common cause of morbidity, and the third leading cause of mortality worldwide, accounting for 5.8% of all deaths [1, 2]. The burden of COPD is higher in the Asia-Pacific region than in industrialised western countries [3]. Smoking and air pollution, either occupational or household from burning biomass, are among the main causes of COPD in the region [4-6].

Acute exacerbations of COPD (AECOPD) contribute to disease progression [1, 7, 8] and significantly impair patients' quality of life [9]. Bacterial and viral infections are recognised as having an important association with AECOPD [7, 10, 11]. Various studies have shown a link between exacerbation and increased prevalence of airway bacteria, including *Haemophilus influenzae* (Hi) and *Moraxella catarrhalis* (Mcat) [12-18], and mixed bacterial and viral infections are frequent [11, 19-23]. In the observational Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) study, bacterial and viral coinfection was more frequent at exacerbation than stable state [17]. Moreover, the odds of experiencing an exacerbation (vs. stable state) was higher when both non-typeable Hi and human rhinovirus (HRV) were detected than when HRV was absent.

There are few data from the Asia-Pacific region on the prevalence of bacterial and viral infections during periods of stable and exacerbated COPD [24]. We conducted a study to estimate the prevalence of bacterial and viral pathogens, overall and by species, with an emphasis on Hi and Mcat, in patients with moderate to very severe COPD in selected Asia-Pacific populations. The incidence rate and duration of AECOPD was estimated, as well as the odds of experiencing an exacerbation in the presence of specific pathogens. The impact of AECOPD on health-related quality of life (HRQOL) was also calculated for the study population.

METHODS

Study design, setting and objectives

This was a prospective, epidemiological study (NCT03151395) of patients with moderate to very severe COPD. All enrolled patients provided written informed consent. The study was conducted between August 2017 and September 2020 in Hong Kong, the Philippines, South Korea and Taiwan and each patient was followed for one year.

Four study visits were scheduled, with additional, unscheduled AECOPD visits performed within 96 hours of the onset of an exacerbation (figure 1). The primary objective was to estimate the proportion of potential bacterial pathogens (by culture-based methods) and viral pathogens (by PCR) in the sputum of patients with stable COPD and at exacerbation over the course of 1 year. PCR estimation of the proportion of bacterial pathogens was assessed as a secondary objective. We also report results for secondary objectives relating to the incidence rate and duration of AECOPD and HRQOL, as well as descriptive results for tertiary objectives relating to the quantity (load) of specific bacteria and viruses. Estimates from a further analysis are also presented for the odds of experiencing an exacerbation (vs. stable state) for the presence, acquisition and apparition of specific pathogens.

Participants

Eligible patients were aged 40 years or older with a confirmed diagnosis of moderate to very severe, stable COPD, with at least one documented exacerbation in the previous year requiring corticosteroids, antibiotics or hospitalisation. COPD was diagnosed based on post-bronchodilator spirometry *i.e.*, ratio of forced expiratory volume in 1 second to forced vital capacity (FEV1/FVC) <0.7, and FEV1 <80% predicted. Disease severity was classified as Global Initiative for Chronic Obstructive Lung Disease (GOLD) grade 2 (moderate), 3 (severe) or 4 (very severe) [1]. Patients were considered as having stable COPD if their last

exacerbation episode had resolved within 30 days before study entry. Eligible patients had either a smoking history of at least 10 pack-years or a history of exposure to biomass smoke for at least 20 years and had to be able to provide a sputum sample.

Patients were excluded if they were diagnosed with a respiratory disorder other than COPD or had a recent (≤3 months) chest X-ray showing evidence of clinically significant abnormalities unrelated to COPD. Other exclusion criteria included α-1 antitrypsin deficiency as an underlying cause of COPD, confirmed or suspected immunosuppression, lung surgery within 12 months before visit 1, chemotherapy within 12 months before visit 1, participation in another clinical study, antibiotic receipt within 1 month of study entry or continuous antibiotic administration (>30 days in total) within 90 days before visit 1 or systemic administration of corticosteroids (>14 days consecutive days) within 90 days before visit 1, and any condition that could interfere with the patient's ability to understand the study procedures. Pregnant women were not enrolled. Contraindications for spirometry testing were additional exclusion criteria.

Exacerbation monitoring and sputum sample collection

The occurrence of a potential exacerbation was monitored using electronic diary cards to record morning symptoms each day. An exacerbation was defined as follows [25]:

a) Worsening of ≥2 major symptoms (dyspnoea, sputum volume, sputum purulence) for ≥2 consecutive days; or

b) Worsening of any major symptom together with any of the following minor symptoms for ≥2 consecutive days: sore throat, cold (nasal discharge and/or nasal congestion), fever (oral temperature ≥37.5°C) without other cause, increased cough, increased wheeze.

The exacerbation end date and severity were determined by a study qualified individual during follow-up phone calls, made at least every 2 weeks until the exacerbation was

resolved. Exacerbation severity was classified as mild if controlled with an increased dosage of regular medications, moderate if requiring treatment with systemic corticosteroids and/or antibiotics, and severe if requiring hospitalisation.

Sputum samples were collected at all study visits (stable state and AECOPD) and were spontaneous or induced (using saline solution), as per the investigator's judgement. If an exacerbation occurred when a stable-state visit was planned, the visit was re-scheduled for when the patient had recovered. Spontaneous self-collection of sputum samples at the patient's home was allowed (only during exacerbation and if the patient needed an antibiotic dose before the AECOPD visit) but there were no cases of self-collection at home during the study. Patients were instructed not to take an antibiotic before study visits. However, in some instances, antibiotic treatment was already initiated. Data on the use of antibiotics before sputum collection were recorded at each study visit and at exacerbation.

Laboratory assays

Sputum samples were diluted in dithiothreitol (DTT), processed and cultured within 6 hours of collection for microbiology testing. Any remaining sample was stored at -70/80°C before shipment to the central study laboratory (Q^2 Solutions, Singapore) for additional testing. Sputum sample quality was assessed by Gram stain, with no rejection criteria.

Bacterial pathogens were identified by standard bacteriological culture methods on fresh sputum samples at study site laboratories, according to each laboratory's routine methods. Bacterial species identified included Hi, Mcat, *S. pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Hi isolates were further analysed by PCR assay to identify non-typeable Hi or other *Haemophilus* species.

For PCR, nucleic acids were extracted using the MagNA Pure 96 equipment (Roche

Diagnostics) with the DNA and Viral NA Large Volume Kit (Roche Diagnostics) and the pathogen universal protocol, as per the manufacturer's instructions. The presence of bacterial pathogens was assessed by PCR assay of frozen DTT-treated sputum samples in the central laboratory using two triplex real-time PCR assays; one quantitative, identifying Hi, Mcat, and *S. pneumoniae*, and the other qualitative, identifying *S. pyogenes*, *S. aureus* and *P. aeruginosa*, as described previously [17] and summarised in the supplementary material. Viral pathogens were identified using a qualitative nucleic acid multiplex test and real-time PCR, as summarised in the supplementary material. Viral pathogens identified included HRV, respiratory syncytial virus, parainfluenza virus, enterovirus, metapneumovirus, influenza virus, adenovirus, bocavirus and coronavirus.

The concentration of bacterial or viral DNA (copies/mL) in each sample was inferred from a calibration curve made of serial dilutions of a plasmid containing the sequences targeted by the assay and converted from copies/PCR to copies/mL of DTT-treated sputum samples.

HRQOL assessment

Patients completed a self-administered COPD assessment test (CAT) at every study visit and self-administered St. George's Respiratory Questionnaire for COPD patients (SGRQ-C) at each of the three scheduled study visits. For each questionnaire, higher scores reflect worse health status [26, 27].

Statistical analysis

The aim was to enrol approximately 200 patients. Assuming that approximately 90% of these patients would have on average one AECOPD per year, of which approximately 80% would be able to provide one AECOPD sputum sample, ≈140 AECOPD sputum samples would be obtained. Assuming 20% of these samples were positive for Hi and Mcat, the exact 95% confidence interval (CI) around this proportion was estimated as 13.7–27.6%, corresponding

to 19 to 39 positive sputum samples, which was considered appropriate to address the study objectives.

The study objectives were assessed on eligible patients who fulfilled screening criteria and completed at least visit 1. Proportions of sputum samples positive for bacterial and viral pathogens and 95% CIs were calculated at stable and exacerbation visits, overall and for each species, using a generalised estimating equations (GEE) model, assuming a binomial distribution for the response variable with logit as the link function and an exchangeable correlation structure to account for within-patient correlations. AECOPD incidence rates and 95% CIs were estimated using a generalised linear model (GLM), assuming a negative binomial distribution for the response variable with logarithm as the link function and the logarithm of follow-up time as an offset variable. If the negative binomial model did not converge due to underdispersion of data, a Poisson model was used to obtain both incidence rates and their 95% CIs. Incidence rates are presented as average number of exacerbations per person per year.

The odds of experiencing an exacerbation (vs. being in a stable state) was calculated for 1) patients with sputum positive for Hi, Mcat or HRV vs. those with negative sputum; 2) patients with sputum positive for Hi, Mcat or HRV for the first time during the study (acquisition) vs. those without acquisition; and 3) patients with sputum positive for Hi, Mcat or HRV who were negative at the previous visit (apparition) vs. those without apparition. To obtain the odds ratios (ORs) associated with these three analyses, a conditional logistic regression model, stratified by patient, was fitted. The model included the pathogen (positive or negative) and season (high season, October–March; low season, April–September) as independent variables.

Descriptive statistics were used to summarise demographic and disease characteristics and HRQOL scores. SAS® Drug Development was used for all statistical analyses.

RESULTS

Patients and sputum samples

Of 230 patients screened, 197 (85.7%) were enrolled and completed at least one visit; 182 (92.4%) completed the study (figure 2). In total, 983 sputum samples were obtained, 226 during AECOPD visits. Most samples were from the Philippines, with the fewest from Hong Kong (figure 2).

The mean age at screening was 68.5 years (range 67.3–70.4 years across countries; figure 3), 94.9% were male and patients had either East Asian (64%) or Southeast Asian ethnicity (36%). In the year before study enrolment, 129 (65.5%) patients had one exacerbation, 18.8% had two, 8.6% had three, 2.5% had four, and 4.6% had more than four exacerbations (figure 3).

AECOPD rate and characteristics

Overall, 227 exacerbations were recorded: 21 were mild, 182 moderate and 24 severe. The estimated yearly incidence rate of confirmed AECOPD (defined in table 1 footnote) was 1.27 per patient overall and increased with increasing GOLD grade at screening (table 1). The mean duration of exacerbations was overall 14.3 days (standard deviation [SD] 9.0), 20.3 days (SD 11.1) for mild, 12.6 days (SD 6.3) for moderate and 21.5 days (SD 16.5) for severe exacerbations.

Prevalence of bacterial and viral species

The culture and PCR results for percentages of sputum samples positive for specific bacterial species differed overall and by country (table 2; supplementary tables 1 and 2). Overall, the bacterial species detected most frequently by culture at stable state and exacerbation were *K. pneumoniae*, *P. aeruginosa* and Hi (table 2; supplementary figure 1). Hi was shown by subsequent PCR analysis to be mostly non-typeable (supplementary table 1). With PCR

detection, the most prevalent bacteria detected were Hi, *S. pneumoniae* and *P. aeruginosa* at stable state and Hi, Mcat and *S. pneumoniae* at exacerbation (table 2, figure 4). *K. pneumoniae* presence was not assessed by PCR. The differences between culture and PCR detection results are likely to be due to better specificity and sensitivity with PCR than with culture [12, 17, 28, 29] and differences in routine culture-based methods used in this study between local laboratories. We therefore focus on the bacterial species results from the PCR assays, which were well characterised and conducted centrally.

The analysis of prevalence data showed the proportion of sputum samples positive by PCR for any bacterial pathogen was higher at AECOPD visits than at stable-state visits (74.7% vs 67.0%, respectively), most notably for Mcat (26.8% in exacerbation vs 13.7% in stable state) (table 2, figure 4). The prevalence of Hi by PCR was similar between stable-state and exacerbation samples, overall and in each country (supplementary table 2). Sample positivity for Hi in stable-state samples ranged from 28.9% (Taiwan) to 57.1% (Hong Kong), and from 16.2% (Taiwan) to 58.8% (Hong Kong) in exacerbation samples. Mcat prevalence in stable-state samples ranged from 4.3% (Hong Kong) to 20.8% (Philippines), and from 18.9% (Taiwan) to 35.4% (Korea) in exacerbation samples. The prevalence of other bacterial species tended to be similar between stable state and exacerbation (figure 4, supplementary table 2).

Viral prevalence was low, other than for HRV (supplementary table 3). The proportion of sputum samples positive for viral pathogens was lower in stable-state samples than at exacerbation for HRV (8.1% and 16.6%, respectively), influenza virus (0.7%, 8.2%), coronavirus (2.6%, 5.7%) and parainfluenza virus (0.7%, 4.7%).

Analysis of bacterial load by quantitative PCR showed an approximate two-fold increase in mean load value for Hi at AECOPD visits (3.6×10^8 copies/mL of sputum; standard deviation [SD] 11.3×10^8) vs stable state (1.6×10^8 copies/mL; SD 5.4×10^8) and no difference between visit types for Mcat and *S. pneumoniae* (supplementary table 4). For HRV, there was

an approximate two-fold increase in mean load at AECOPD visits (7.0 x 10^6 copies/mL; SD 18.7 x 10^6) vs stable state (3.1 x 10^6 copies/mL; SD 13.9 x 10^6) (supplementary table 4).

Analysis of the prevalence of bacterial pathogens in sputum samples according to the use of antibiotics vs no antibiotics before sputum sample collection suggested antibiotic administration had no major impact, although prior antibiotic administration was infrequent (supplementary table 5).

Odds of exacerbation for pathogen presence, acquisition and apparition

Using the PCR assay results, the odds of experiencing an exacerbation (vs. stable state) was calculated according to pathogen detected as: 1) presence vs. absence; 2) acquisition (detection for the first time during the study) vs. no acquisition; and 3) apparition (detection after negative sputum sample at previous visit) vs. no apparition. The OR for experiencing an exacerbation rather than being in stable state was significant for Hi, Mcat and HRV detection for all three analyses (figure 5). Specifically, the odds of an exacerbation when there was presence, acquisition and apparition of Hi were 2.20 (95% CI 1.26–3.86; p=0.006), 2.43 (1.11–5.35; p=0.027) and 2.32 (1.20–4.46; p=0.012) times higher compared to no presence, no acquisition and no apparition of Hi, respectively. For Mcat, the ORs were 2.24 (95% CI 1.30–3.88; p=0.004), 5.47 (2.16–13.86; p<0.001) and 3.45 (1.71–6.98; p<0.001) for presence, acquisition and apparition, respectively. For HRV, the ORs were 2.12 (95% CI 1.15–3.91; p=0.017), 2.22 (1.09–4.55; p=0.029) and 2.09 (1.11–3.91; p=0.022) for presence, acquisition and apparition, respectively.

HRQOL scores

Mean CAT scores were 12.66 at stable-state visits, approximately 20 at mild and moderate AECOPD visits and 26 at severe AECOPD visits (figure 6). Stable-state SGRQ-C scores remained constant over time (figure 6). Total CAT and SGRQ-C scores at the final scheduled

visit were higher than those at the first scheduled visit for patients with more than three exacerbations compared with those experiencing fewer exacerbations during the 1-year follow-up (figure 6).

DISCUSSION

In this 1-year prospective follow-up study, we evaluated selected bacteria and viruses detected by culture-based methods and PCR in sputum samples from patients with COPD during periods of stable disease and exacerbation. We present findings from an analysis of the prevalence of each species, and an analysis of the odds of experiencing an exacerbation (vs. stable-state COPD) for the presence, acquisition and apparition of specific pathogens.

Our results indicate higher sensitivity of PCR than culture assay in the identification of airway bacteria, as reported in AERIS and other studies of respiratory bacteria in patients with COPD [12, 17, 28, 29], with PCR positivity rates between five- and 15-fold higher than culture positivity for Hi and Mcat. As well as Hi and Mcat, other bacterial species identified most frequently by PCR assay were *S. pneumoniae* and *P. aeruginosa* at stable state and exacerbation, which is consistent with previous studies of patients with COPD [7, 23].

The proportion of sputum samples positive by PCR for any bacterial pathogen was higher at AECOPD visits than at stable state. However, examination of trends by species indicated a clear difference for Mcat only. The prevalence of all other species, including Hi, was similar in stable disease and at exacerbation. Analysis of detected viruses showed HRV was the most prevalent species and was overall twice as prevalent in AECOPD as in stable-state COPD. Another notable observation was that influenza virus was overall 12 times more prevalent in AECOPD compared with stable state. This aligns with previous reports of sharp increases in viral prevalence from stable-state COPD to exacerbation state [7, 23]. Moreover, the OR for exacerbation (vs. stable state) was significant when there was presence, acquisition or

apparition of HRV, and for presence, acquisition and apparition of both Hi and Mcat. The OR results therefore indicate a potential involvement of Hi, Mcat and HRV in triggering an exacerbation. This was also suggested by results from the AERIS study, in which a significant OR for AECOPD occurrence was found when Mcat was detected, and a significant interaction was detected between non-typeable Hi and HRV presence and AECOPD risk [17].

Patients with severe and very severe COPD at screening had a higher incidence of exacerbations during the study than patients with moderate COPD, with exacerbation incidence rates ranging from under one per year (moderate, GOLD grade 2) to almost two per year (very severe, GOLD grade 4). A similar trend, but with lower AECOPD incidence rates, was reported in a retrospective analysis of 886 patients with COPD in the Netherlands, in which exacerbation incidence rates were two-fold higher in patients with very severe COPD than in those with moderate COPD [30]. We also found evidence of worsening general health status during the study that was more pronounced in patients experiencing more frequent exacerbations. Similar trends were observed in a meta-analysis of randomised trials involving over 18,000 patients with COPD, which showed incremental deteriorations in HRQOL with every moderate or severe COPD exacerbation over a 1-year period [31].

The patient population in this study was predominantly male and was of either East Asian or Southeast Asian heritage, potentially limiting the generalisability of our findings, although this population was selected to address a data gap in the literature for patients with COPD. Another possible limitation is that, because of the smaller sample size, data from the Hong Kong cohort may be less robust. Also, as already highlighted, interpretation of the bacterial culture results is limited by the range of culture-based methods used in local laboratories. Finally, bacterial load may have a role in changing the clinical status of patients with COPD [32], but this was not analysed fully in this study. An important strength of the study is its

prospective, longitudinal design that allowed collection of multiple sputum samples during periods of stable disease and exacerbation in the same individuals. All datasets and statistical analyses underwent double independent programming to ensure high quality data outputs.

Conclusion

This study showed a high prevalence of Hi and Mcat bacteria in both stable and AECOPD states among patients with COPD in the Asia-Pacific region. Viral prevalence other than HRV was low, although influenza was markedly increased at exacerbation. The presence of Hi, Mcat and HRV significantly increased the odds of an exacerbation, providing further evidence of the potentially important role for these pathogens in exacerbations. Our results also confirm the negative impact of more frequent exacerbations on patients' quality of life.

Disclosures

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The protocols and associated documents were reviewed and approved by the ethics committee of each participating centre. All participants provided written informed consent before study entry.

Consent for publication

Not applicable.

Availability of data and materials

Anonymised individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

Competing interests

Laura Taddei, Ludovic Malvaux and Ashwani Kumar Arora are employed by the GSK group of companies. Laura Taddei and Ludovic Malvaux hold shares in the GSK group of companies. Lucio Malvisi was employed by the GSK group of company at the time the study was conducted. Laura Taddei, Ludovic Malvaux, Ashwani Kumar Arora and Lucio Malvisi declare no other financial and non-financial relationships and activities. David Hui, Ronnie Z. Samoro, Sang Haak Lee, Yiu Cheong Yeung and Yu-Chih Liu declare no financial and non-financial relationships and activities and no conflicts of interest.

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Authors' contributions

Laura Taddei, Ludovic Malvaux and Ashwani Kumar Arora were involved in the study conception and design. Lucio Malvisi was involved in the laboratory conception and data generation. All authors were involved in acquisition and generation of data and/or performed the study. Ashwani Kumar Arora, Ludovic Malvaux, Laura Taddei and Lucio Malvisi were involved in data analysis and data interpretation. All authors contributed substantially to the development of the manuscript and had full access to the data. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article.

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Table 1. Estimated incidence rate of AECOPD during the 1-year follow-up

	Incidence rate* of AECOPD (95% CI)	
GOLD grade at screening (N=197)	**Confirmed (n=249)**	**Confirmed + potential (n=256)**
All grades	1.27 (1.04–1.54)	1.30 (1.07–1.58)
GOLD 2 (n=79)	0.78 (0.54–1.14)	0.81 (0.56–1.16)
GOLD 3 (n=99)	1.51 (1.17–1.94)	1.56 (1.20–2.01)
GOLD 4 (n=19)	1.99 (1.37–2.89)	1.99 (1.37–2.89)

*Mean number of exacerbations per year from the negative binomial model (or Poisson model in cases of underdispersion)

Notes: (a) Confirmed AECOPD: exacerbation events plus missed exacerbation events (missed exacerbations correspond to all morning alerts confirmed by a phone call, as well as cases with no morning alert for which there was no site visit but for which AECOPD medical records were available). (b) Potential AECOPD: all morning alerts confirmed by a phone call for which there was no site visit and for which no medical records were available. (c) GOLD severity grading: grade 2 (moderate airflow limitation), 3 (severe airflow limitation), 4 (very severe airflow limitation).

AECOPD, acute exacerbations of chronic obstructive pulmonary disease; CI, confidence interval; GOLD, Global Initiative for Chronic Obstructive Lung Disease; n, number (of patients per given category)

Table 2. Bacterial pathogens detected in sputum samples by culture or PCR throughout study follow-up, during periods of stable disease (scheduled visits) and at exacerbation (AECOPD visits)

	Percentage of bacteria-positive sputum samples (95% CI)			
	Culture		PCR	
Bacterial species	**Scheduled visits (N=554)**	**AECOPD visits (N=209)**	**Scheduled visits (N=546)**	**AECOPD visits (N=194)**
Any	74.2 (70.4–77.7)	78.0 (72.1–83.2)	67.0 (63.0–70.9)	74.7 (68.3–80.5)
H. influenzae	6.7	8.1	42.9 (38.7–47.0)	39.7 (33.0–46.7)
M. catarrhalis	0.9 (0.3–1.9)	3.3 (1.5–6.4)	13.7 (11.0–16.8)	26.8 (20.9–33.3)
S. pneumoniae	2.5 (1.4–4.1)	2.4 (0.9–5.1)	16.8 (13.9–20.1)	19.1 (14.0–25.0)
S. pyogenes	NA	NA	0.4 (0.1–1.1)	1.0 (0.2–3.1)
S. aureus	3.1 (1.8–4.7)	3.3 (1.5–6.4)	10.6 (8.2–13.4)	9.3 (5.7–13.9)
P. aeruginosa	8.5 (6.4–11.0)	14.4 (10.0–19.5)	16.3 (13.4–19.6)	16.5 (11.7–22.1)
K. pneumoniae	26.4 (22.8–30.1)	24.9 (19.3–31.0)	NA	NA
A. baumannii	2.2 (1.2–3.6)	4.3 (2.1–7.6)	NA	NA
Other	47.7 (43.5–51.8)	45.0 (38.3–51.8)	NA	NA

AECOPD, acute exacerbations of chronic obstructive pulmonary disease; CI, confidence interval; N, number of patients per given category; NA, not assessed

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Figures

Figure 1. Study design

AECOPD, acute exacerbations of chronic obstructive pulmonary disease; ATS-DLD-078, American Thoracic Society and National Heart and Lung Institute Division of Lung Disease Respiratory Questionnaire; BS, blood sample; CAT, COPD assessment tool; e-DC, training and use of electronic diary card; M. month; SGRQ-C, St. George's Respiratory Questionnaire for COPD; SP, sputum sample; spiro, spirometry; V, visit.

* Sputum could be collected spontaneously or could be induced, as per the investigator's judgement

** Follow up phone calls were made at least every 2 weeks until the exacerbation was resolved.

Notes: (a) The four scheduled, stable-state COPD study visits were as follows: the initial screening visit (V0, pre-month 0), the baseline visit at month 0 (V1), the visit at month 6 (V2), and the final study visit at the end of the follow-up period at month 12 (V3). Additional unscheduled AECOPD visits were performed per protocol. (b) From screening visit to visit 1, eligible patients were trained in the use of the e-DC given to them at screening. At all study visits, sputum samples were collected for culture and polymerase chain reaction analyses for pathogen identification, and CAT and SGRQ-C questionnaires were also completed.

Figure 2. Patient disposition flow diagram

AECOPD, acute exacerbations of chronic obstructive pulmonary disease; n, number (of patients per given category); SAE, serious adverse event

*****Other: patient experienced AECOPD during their screening visit or other reason not specified

**Reasons for discontinuation are in parentheses: consent withdrawal due to a SAE (SAE), consent withdrawal for another reason (consent), lost to follow-up before the final study visit (lost to FU), other (other).

†Total sputum samples included in the full analysis set = samples obtained at all study visits

(scheduled visits and unscheduled AECOPD visits)

††Sputum samples excluded from the full analysis set due to (1) protocol deviations, (2) patients had not recovered at a scheduled visit, (3) samples taken from exacerbations occurring prior to visit 1.

Figure 3. Patient baseline characteristics

%: number of patients in a given category / total number of patients *100; GOLD, Global Initiative for Chronic Obstructive Lung Disease; SD, standard deviation; y, year(s).
Notes: a) GOLD severity grading: grade 2 (moderate airflow limitation), 3 (severe airflow limitation), 4 (very severe airflow limitation). B) AECOPD severity grading: mild - controlled with increased dosage of regular medications; moderate - required treatment with systemic corticosteroids and/or antibiotics; severe - required hospitalisation.

Figure 4. Percentage (95% confidence intervals) of sputum samples positive for bacteria by PCR analysis, by type of visit: scheduled or unscheduled AECOPD visit. Data from the culture analysis are provided in supplementary figure 1.

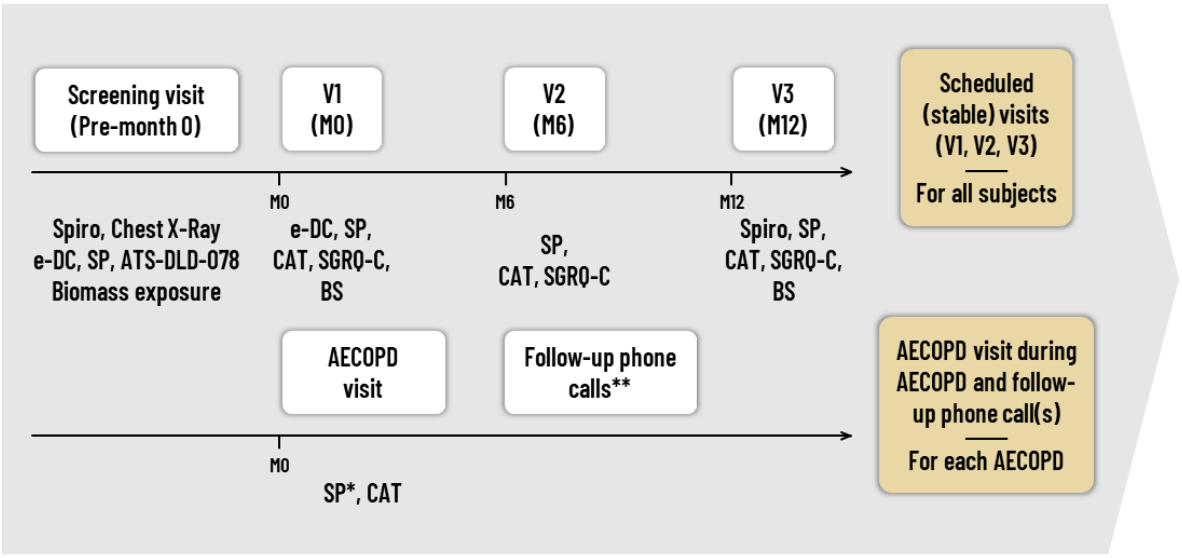
AECOPD, acute exacerbations of chronic obstructive pulmonary disease; CI, confidence interval.

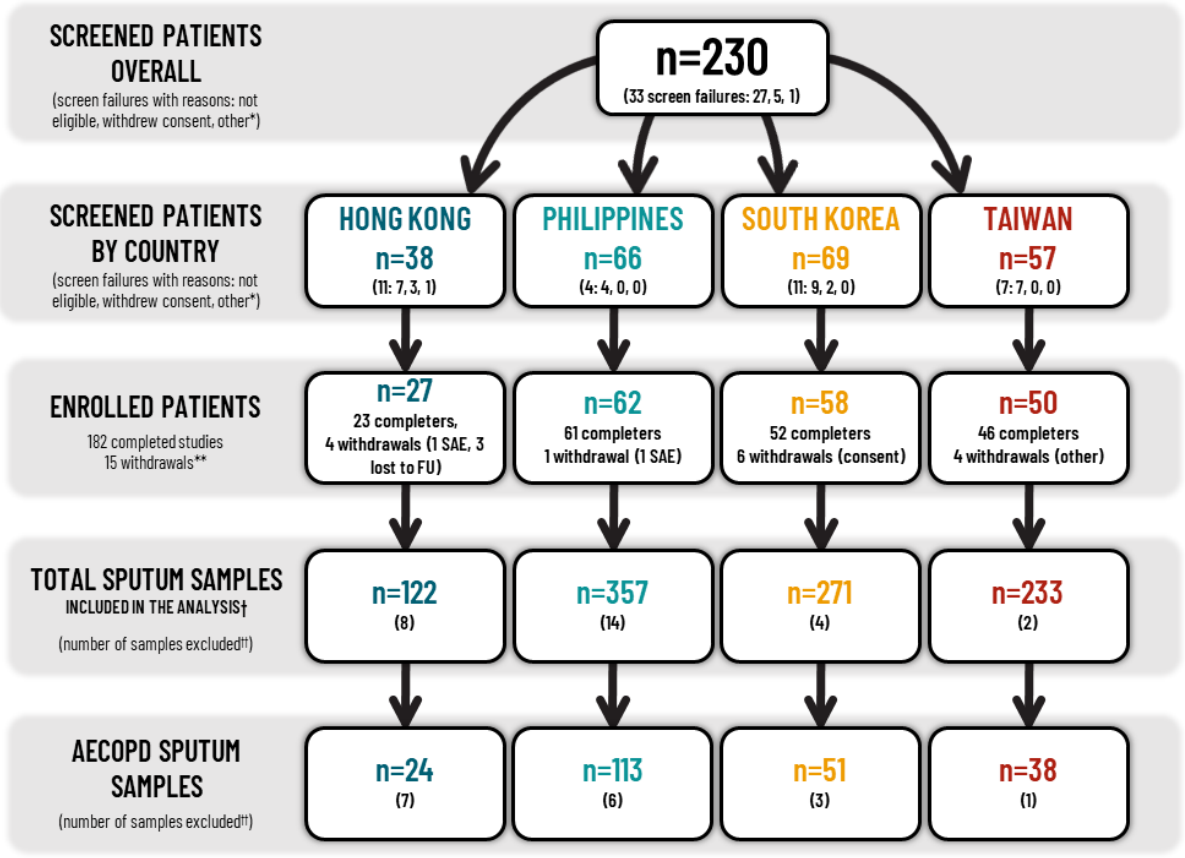
Figure 5. Odds ratios (with 95% confidence intervals) for effect of the presence, acquisition (sputum positive for the first time during the study) or apparition (sputum positive after negative sputum sample at previous visit) of *Haemophilus influenzae*, *Moraxella catarrhalis* or HRV detected by PCR on the odds of experiencing AECOPD rather than being in stable state.

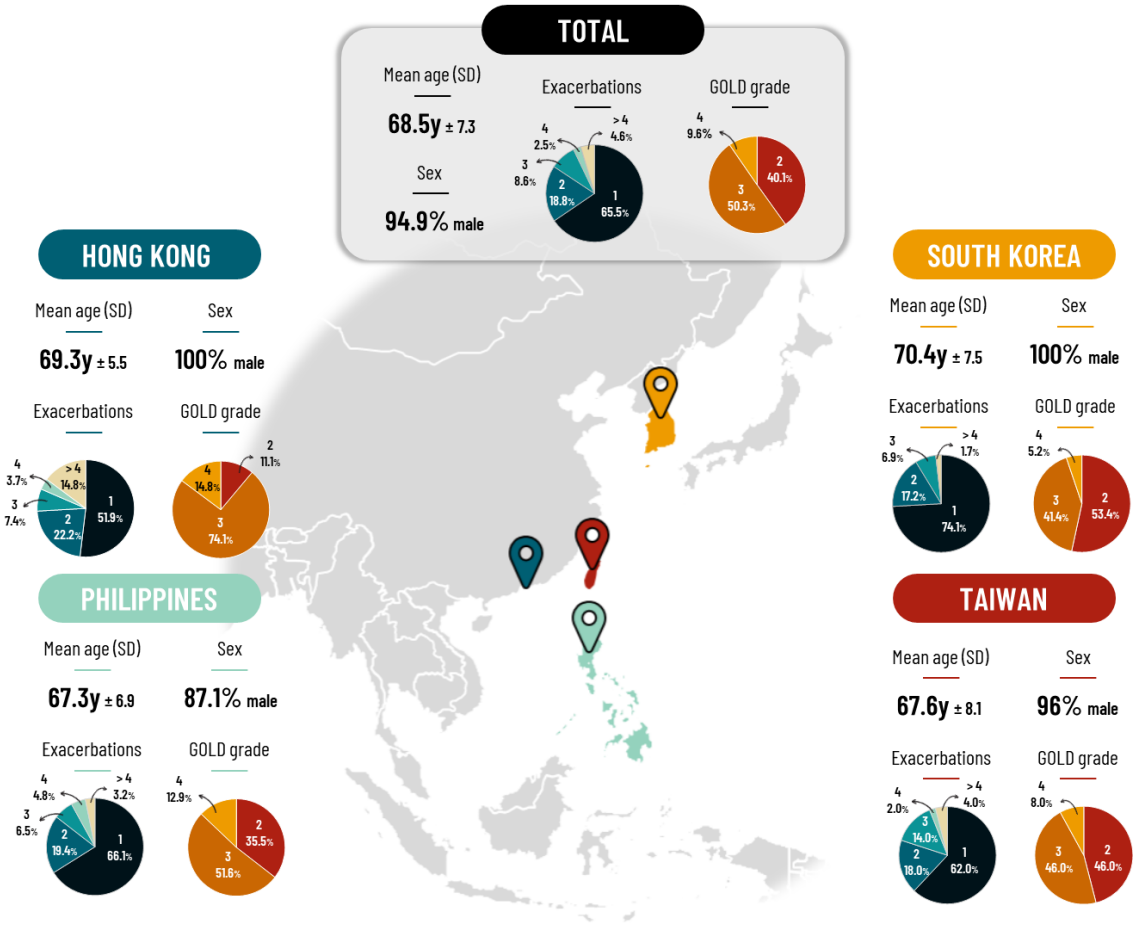
AECOPD, acute exacerbations of chronic obstructive pulmonary disease; CI, confidence interval; HRV, human rhinovirus; OR, odds ratio

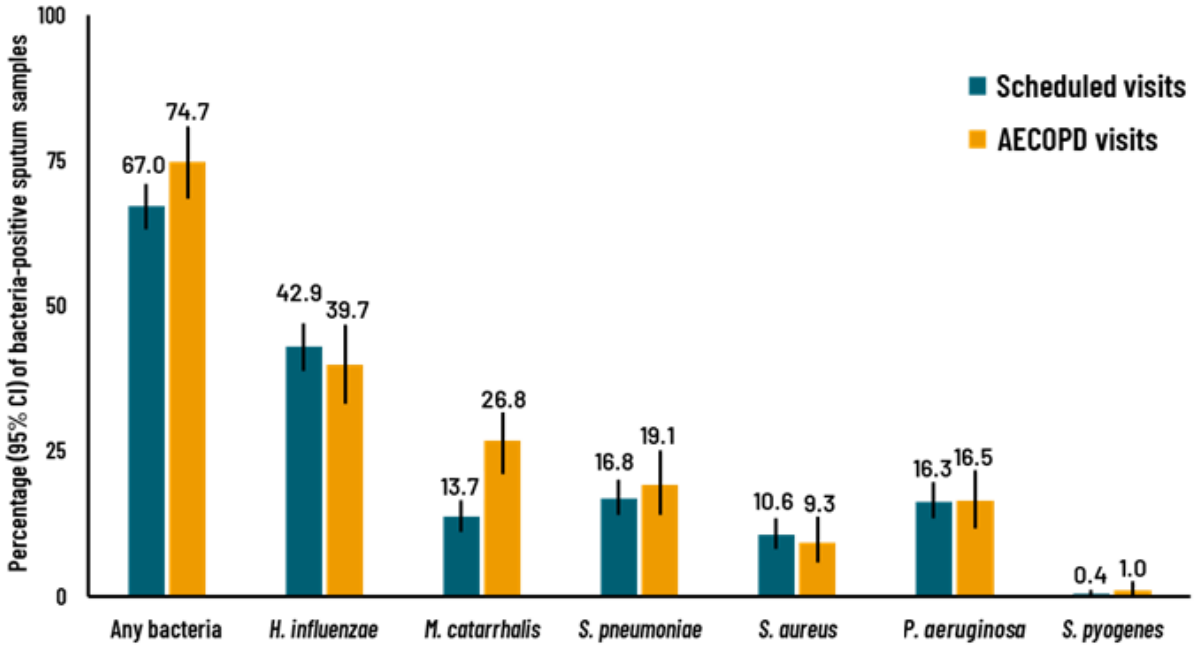
Figure 6. CAT and SGRQ-C scores by (A) visit and (B) by total score difference from the first to final visit according to number of exacerbations experienced during the 1-year follow-up

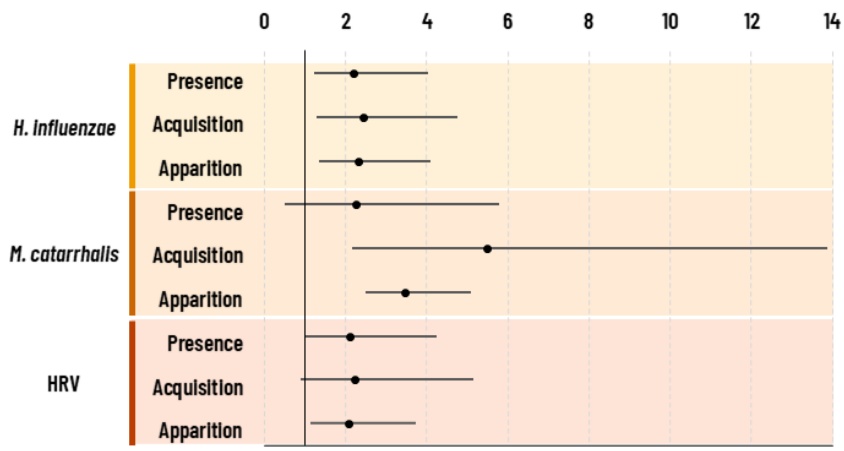
AECOPD, acute exacerbations of COPD; CAT, COPD assessment test; COPD, chronic obstructive pulmonary disease; SGRQ-C, St. George's Respiratory Questionnaire for COPD


Screening visit
(Pre-month 0)
V1
(M0)
V2
(M6)
V3
(M12)
Scheduled (stable) visits (V1, V2, V3)
For all subjects
M0
M6
M12
Spiro, Chest X-Ray
e-DC, SP, ATS-DLD-078
Biomass exposure
e-DC, SP,
CAT, SGRQ-C,
BS
SP,
CAT, SGRQ-C
Spiro, SP,
CAT, SGRQ-C,
BS
AECOPD visit
Follow-up phone calls**
AECOPD visit during AECOPD and follow-up phone call(s)
For each AECOPD
M0
SP*, CAT


SCREENED PATIENTS OVERALL
(screen failures with reasons: not eligible, withdrew consent, other*)
n=230
(33 screen failures: 27, 5, 1)
SCREENED PATIENTS BY COUNTRY
(screen failures with reasons: not eligible, withdrew consent, other*)
HONG KONG
n=38
(11: 7, 3, 1)
PHILIPPINES
n=66
(4: 4, 0, 0)
SOUTH KOREA
n=69
(11: 9, 2, 0)
TAIWAN
n=57
(7: 7, 0, 0)
ENROLLED PATIENTS
182 completed studies
15 withdrawals**
n=27
23 completers,
4 withdrawals (1 SAE, 3 lost to FU)
n=62
61 completers
1 withdrawal (1 SAE)
n=58
52 completers
6 withdrawals (consent)
n=50
46 completers
4 withdrawals (other)
TOTAL SPUTUM SAMPLES
INCLUDED IN THE ANALYSIS†
(number of samples excluded††)
n=122
(8)
n=357
(14)
n=271
(4)
n=233
(2)
AECOPD SPUTUM SAMPLES
(number of samples excluded††)
n=24
(7)
n=113
(6)
n=51
(3)
n=38
(1)

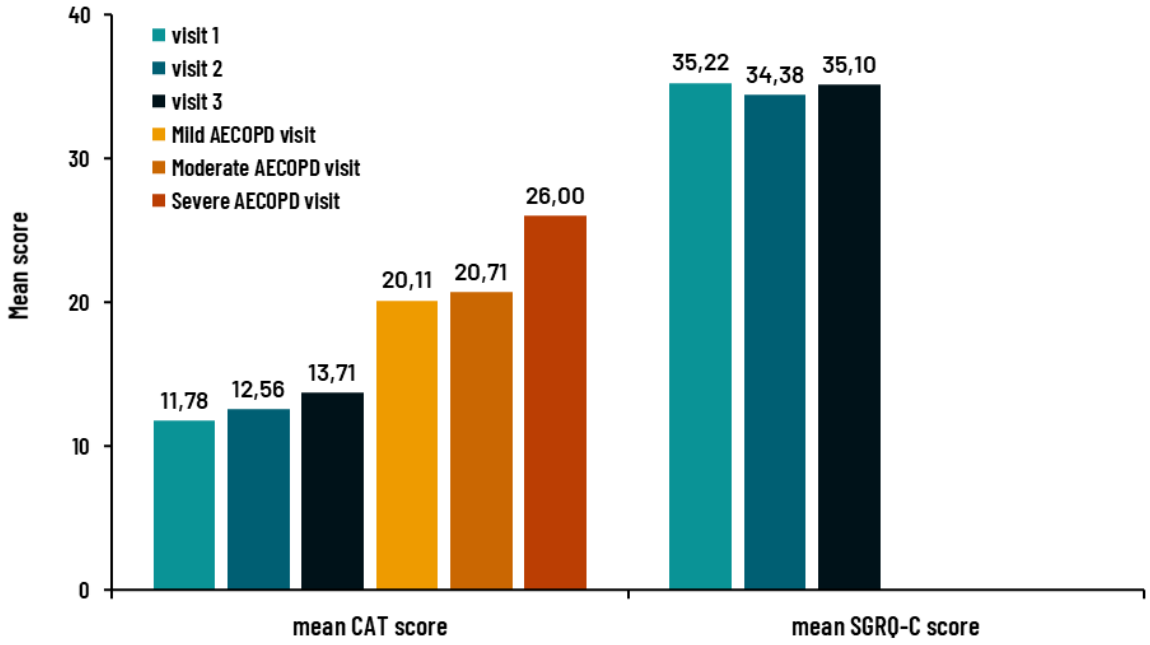

TOTAL
Mean age (SD)
68.5y ± 7.3
Sex
94.9% male
Exacerbations
1 65.5%
2 18.8%
3 8.6%
4 2.5%
>4 4.6%
GOLD grade
2 40.1%
3 50.3%
4 9.6%
HONG KONG
Mean age (SD)
69.3y ± 5.5
Sex
100% male
Exacerbations
1 51.9%
2 22.2%
3 7.4%
4 3.7%
>4 14.8%
GOLD grade
2 11.1%
3 74.1%
4 14.8%
PHILIPPINES
Mean age (SD)
67.3y ± 6.9
Sex
87.1% male
Exacerbations
1 66.1%
2 19.4%
3 6.5%
4 4.8%
>4 3.2%
GOLD grade
2 35.5%
3 51.6%
4 12.9%
SOUTH KOREA
Mean age (SD)
70.4y ± 7.5
Sex
100% male
Exacerbations
1 74.1%
2 17.2%
3 6.9%
>4 1.7%
GOLD grade
2 53.4%
3 41.4%
4 5.2%
TAIWAN
Mean age (SD)
67.6y ± 8.1
Sex
96% male
Exacerbations
1 62.0%
2 18.0%
3 14.0%
4 2.0%
>4 4.0%
GOLD grade
2 46.0%
3 46.0%
4 8.0%


Percentage (95% CI) of bacteria-positive sputum samples
100
75
50
25
0
Scheduled visits
AECOPD visits
67.0
74.7
42.9
39.7
13.7
26.8
16.8
19.1
10.6
9.3
16.3
16.5
0.4
1.0
Any bacteria
H. influenzae
M. catarrhalis
S. pneumoniae
S. aureus
P. aeruginosa
S. pyogenes

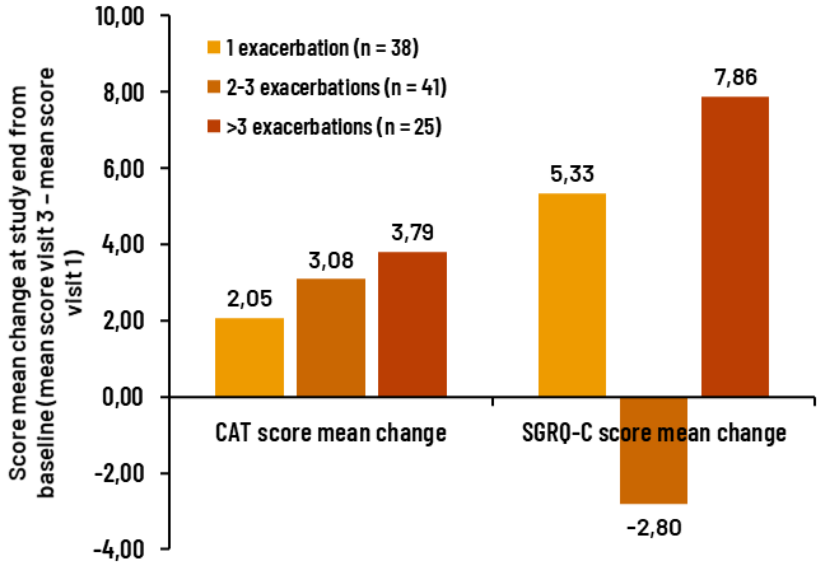


		Odds ratio (95% CI)	*p*
H. influenzae	Presence	2.20 (1.26–3.86)	0.006
	Acquisition	2.43 (1.11–5.35)	0.027
	Apparition	2.32 (1.20–4.46)	0.012
M. catarrhalis	Presence	2.24 (1.30–3.88)	0.004
	Acquisition	5.47 (2.16–13.86)	<0.001
	Apparition	3.45 (1.71–6.98)	<0.001
HRV	Presence	2.12 (1.15–3.91)	0.017
	Acquisition	2.22 (1.09–4.55)	0.029
	Apparition	2.09 (1.11–3.91)	0.022

A) CAT and SGRQ-C mean scores



B) CAT and SGRQ-C total score difference from first to final visit



Bacteria/viruses detected in moderate to very severe COPD and AECOPD in Asia-Pacific

Laura Taddei, Lucio Malvisi, David S. Hui, Ludovic Malvaux, Ronnie Z. Samoro, Sang Haak Lee, Yiu Cheong Yeung, Yu-Chih Liu, Ashwani Kumar Arora

Supplement

PCR methods

A triplex real-time quantitative PCR assay was used for the detection and quantification of the lipo-oligosaccharide glycosyltransferase encoding gene (*lgtC*) of *Haemophilus influenzae*, the CopB outer membrane protein encoding gene (*copB*) of *Moraxella catarrhalis* and the autolysin encoding gene (*lytA*) of *Streptococcus pneumoniae*. The presence of *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* was determined using a qualitative triplex real-time PCR assay targeting conserved regions of the CDS23 gene, the clumping factor A encoding gene (*clfA*) and the GDP mannose dehydrogenase encoding gene (*algD*), respectively.

Isolates initially identified as *H. influenzae* by bacteriological methods were later retested by PCR, targeting the glycosyltransferase (*lgtC*) and outer membrane protein P6 (P6) encoding genes [1] to differentiate *H. influenzae* from *H. haemolyticus*.

The Seegene *Allplex* Respiratory Panel assays (Panels 1, 2 and 3) are qualitative multiplex one-step RT-PCR assays intended for the simultaneous detection and identification of multiple respiratory virus nucleic acids in respiratory specimens [2,3]. Panel 1 detects influenza A, including subtypes of influenza A (H1 and H3), and distinguishes between 2009 H1N1 and other H1N1 (seasonal) strains, influenza B and respiratory syncytial virus; panel 2 detects adenovirus, human metapneumovirus, parainfluenza virus 1–4 and enterovirus; panel 3 detects coronavirus (OC43, 229E, NL63), rhinovirus and bocavirus.

A quantitative real-time PCR assay was used for the detection and quantification of a fragment of a conserved region of the 5' noncoding region of rhinovirus [4] in samples displaying a positive signal for rhinovirus/enterovirus by *Allplex* assay.

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Supplementary Table 1. Bacterial pathogens detected by culture throughout the entire study follow-up period in sputum samples during periods of stable disease and during AECOPD visits, overall and by country.

	Percentage of pathogen-positive sputum samples (95% CI)*									
	All		Hong Kong		Philippines		South Korea		Taiwan	
	Scheduled visits N=554	AECOPD visits N=209	Scheduled visits N=70	AECOPD visits N=17	Scheduled visits N=179	AECOPD visits N=107	Scheduled visits N=161	AECOPD visits N=48	Scheduled visits N=144	AECOPD visits N=37
Any	74.2 (70.4-77.7)	78.0 (72.1-83.2)	97.1 (91.4-99.5)	94.1 (76.6-99.7)	87.7 (82.4-92.0)	90.7 (84.2-95.2)	48.4 (40.8-56.1)	56.2 (42.2-69.7)	75.0 (67.5-81.6)	62.2 (46.1-76.6)
Hi	6.7	8.1	5.7	29.4	5.6	6.5	8.7	6.3	6.3	5.4
NTHi	6.3	7.7	5.7	29.4	5.0	6.5	8.7	4.2	5.6	5.4
Non-Hi	0.4	0.5	0.0	0.0	0.6	0.0	0.0	2.1	0.7	0.0
M. catarrhalis	0.9 (0.3-1.9)	3.3 (1.5-6.4)	0.0 (0.0-5.1)	0.0 (0.0-19.5)	0.0 (0.0-2.0)	0.9 (0.1-4.1)	2.5 (0.8-5.7)	4.2 (0.7-12.3)	0.7 (0.0-3.0)	10.8 (3.5-23.4)
S. pneumoniae	2.5 (1.4-4.1)	2.4 (0.9-5.1)	1.4 (0.1-6.1)	0.0 (0.0-19.5)	0.6 (0.0-2.4)	0.0 (0.0-3.4)	6.2 (3.2-10.6)	8.3 (2.7-18.3)	1.4 (0.2-4.2)	2.7 (0.2-11.4)
S. aureus	3.1 (1.8-4.7)	3.3 (1.5-6.4)	0.0 (0.0-5.1)	0.0 (0.0-19.5)	2.2 (0.7-5.1)	1.9 (0.3-5.7)	0.0 (0.0-2.3)	0.0 (0.0-7.4)	9.0 (5.1-14.4)	13.5 (5.1-26.8)
P. aeruginosa	8.5 (6.4-11.0)	14.4 (10.0-19.5)	4.3 (1.1-10.7)	5.9 (0.3-23.4)	11.7 (7.6-17.0)	19.6 (12.9-27.8)	4.3 (1.9-8.2)	8.3 (2.7-18.3)	11.1 (6.7-16.9)	10.8 (3.5-23.4)
K. pneumoniae	26.4 (22.8-30.1)	24.9 (19.3-31.0)	1.4 (0.1-6.1)	0.0 (0.0-19.5)	38.5 (31.6-45.8)	36.4 (27.7-45.8)	19.9 (14.2-26.5)	12.5 (5.2-23.7)	30.6 (23.4-38.4)	18.9 (8.6-33.4)
A. baumannii	2.2 (1.2-3.6)	4.3 (2.1-7.6)	0.0 (0.0-5.1)	11.8 (2.1-32.1)	2.8 (1.0-5.9)	4.7 (1.7-9.8)	1.2 (0.2-3.8)	0.0 (0.0-7.4)	3.5 (1.3-7.3)	5.4 (0.9-15.8)
Other	47.7 (43.5-51.8)	45.0 (38.3-51.8)	88.6 (79.8-94.6)	70.6 (47.1-88.3)	61.5 (54.2-68.4)	57.0 (47.5-66.1)	23.0 (16.9-29.9)	31.3 (19.4-45.1)	38.2 (30.5-46.3)	16.2 (6.8-30.2)

AECOPD, acute exacerbations of chronic obstructive pulmonary disease; CI, confidence interval; Hi, *Haemophilus influenzae*; N, number of patients per given category; NTHi, non-typeable *Haemophilus influenzae*

*when computed

Supplementary Table 2 Bacterial pathogens detected by PCR throughout the entire study follow-up period in sputum samples collected during periods of stable disease (scheduled visits) and during AECOPD visits, overall and by country.

	Percentage of pathogen-positive sputum samples (95% CI)									
	All		**Hong Kong**		**Philippines**		**South Korea**		**Taiwan**	
	Scheduled visits N=546	**AECOPD visits** N=194	**Scheduled visits** N=70	**AECOPD visits** N=17	**Scheduled visits** N=173	**AECOPD visits** N=92	**Scheduled visits** N=161	**AECOPD visits** N=48	**Scheduled visits** N=142	**AECOPD visits** N=37
Any	67.0 (63.0-70.9)	74.7 (68.3-80.5)	68.6 (57.2-78.6)	88.2 (67.9-97.9)	73.4 (66.5-79.6)	75.0 (65.5-83.1)	70.2 (62.8-76.9)	83.3 (71.2-92.0)	54.9 (46.7-63.0)	56.8 (40.7-71.9)
H. influenzae	42.9 (38.7-47.0)	39.7 (33.0-46.7)	57.1 (45.4-68.3)	58.8 (35.4-79.7)	46.8 (39.5-54.3)	44.6 (34.7-54.8)	44.7 (37.2-52.4)	41.7 (28.4-55.8)	28.9 (21.8-36.7)	16.2 (6.8-30.2)
M. catarrhalis	13.7 (11.0-16.8)	26.8 (20.9-33.3)	4.3 (1.1-10.7)	23.5 (8.0-46.5)	20.8 (15.2-27.3)	26.1 (17.9-35.6)	15.5 (10.5-21.6)	35.4 (22.9-49.5)	7.7 (4.1-12.9)	18.9 (8.6-33.4)
S. pneumoniae	16.8 (13.9-20.1)	19.1 (14.0-25.0)	4.3 (1.1-10.7)	5.9 (0.3-23.4)	20.2 (14.7-26.6)	21.7 (14.2-30.9)	26.7 (20.3-33.9)	27.1 (15.9-40.6)	7.7 (4.1-12.9)	8.1 (2.1-19.7)
S. aureus	10.6 (8.2-13.4)	9.3 (5.7-13.9)	5.7 (1.8-12.8)	5.9 (0.3-23.4)	11.6 (7.4-16.9)	8.7 (4.1-15.6)	9.9 (6.0-15.2)	10.4 (3.9-21.1)	12.7 (7.9-18.8)	10.8 (3.5-23.4)
P. aeruginosa	16.3 (13.4-19.6)	16.5 (11.7-22.1)	15.7 (8.5-25.4)	5.9 (0.3-23.4)	19.7 (14.2-26.0)	16.3 (9.7-24.7)	10.6 (6.4-15.9)	14.6 (6.5-26.3)	19.0 (13.1-26.0)	24.3 (12.5-39.6)
S. pyogenes	0.4 (0.1-1.1)	1.0 (0.2-3.1)	0.0 (0.0-5.1)	0.0 (0.0-19.5)	1.2 (0.2-3.5)	2.2 (0.4-6.6)	0.0 (0.0-2.3)	0.0 (0.0-7.4)	0.0 (0.0-2.6)	0.0 (0.0-9.5)

AECOPD, acute exacerbations of chronic obstructive pulmonary disease; CI, confidence interval; Hi, *Haemophilus influenzae*; N, number of patients per given category; NTHi, non-typeable *Haemophilus influenzae*; PCR, polymerase chain reaction

Supplementary Table 3. Viral pathogens detected by PCR throughout the entire study follow-up period in sputum samples collected during periods of stable disease (scheduled visits) and during AECOPD visits, overall and by country.

	Percentage of pathogen-positive sputum samples (95% CI)									
	All		Hong Kong		Philippines		South Korea		Taiwan	
	Scheduled visits N=546	AECOPD visits N=194	Scheduled visits N=70	AECOPD visits N=17	Scheduled visits N=173	AECOPD visits N=92	Scheduled visits N=161	AECOPD visits N=48	Scheduled visits N=142	AECOPD visits N=37
Any	15.0 (12.2-18.2)	35.6 (29.1-42.5)	12.9 (6.4-22.0)	35.3 (15.8-58.9)	24.3 (18.3-31.0)	40.2 (30.6-50.4)	11.2 (6.9-16.7)	33.3 (21.1-47.3)	9.2 (5.1-14.6)	27.0 (14.6-42.6)
RSV	0.9 (0.3-2.0)	1.0 (0.2-3.1)	0.0 (0.0-5.1)	0.0 (0.0-19.5)	1.7 (0.4-4.4)	1.1 (0.1-4.7)	1.2 (0.2-3.8)	0.0 (0.0-7.4)	0.0 (0.0-2.6)	2.7 (0.2-11.4)
Parainfluenza virus	0.7 (0.2-1.7)	4.7 (2.3-8.2)	0.0 (0.0-5.1)	5.9 (0.3-23.4)	1.2 (0.2-3.5)	2.2 (0.4-6.6)	0.6 (0.0-2.7)	2.1 (0.1-9.0)	0.7 (0.0-3.1)	13.5 (5.1-26.8)
Enterovirus	0.6 (0.1-1.4)	0.0 (0.0-1.9)	0.0 (0.0-5.1)	0.0 (0.0-19.5)	1.7 (0.4-4.4)	0.0 (0.0-3.9)	0.0 (0.0-2.3)	0.0 (0.0-7.5)	0.0 (0.0-2.6)	0.0 (0.0-9.5)
HRV	8.1 (6.0-10.6)	16.6 (11.8-22.3)	8.6 (3.5-16.6)	17.6 (4.7-39.6)	13.9 (9.3-19.5)	18.5 (11.5-27.2)	5.0 (2.3-9.1)	17.0 (8.2-29.4)	4.3 (1.7-8.4)	10.8 (3.5-23.4)
Metapneumovirus	0.0 (0.0-0.7)	0.0 (0.0-1.9)	0.0 (0.0-5.1)	0.0 (0.0-19.5)	0.0 (0.0-2.1)	0.0 (0.0-3.9)	0.0 (0.0-2.3)	0.0 (0.0-7.5)	0.0 (0.0-2.6)	0.0 (0.0-9.5)
Influenza virus	0.7 (0.2-1.7)	8.2 (4.9-12.7)	1.4 (0.1-6.1)	5.9 (0.3-23.4)	0.6 (0.0-2.5)	8.7 (4.1-15.6)	0.6 (0.0-2.7)	8.3 (2.7-18.3)	0.7 (0.0-3.1)	8.1 (2.1-19.7)
Adenovirus	2.0 (1.1-3.4)	4.1 (1.9-7.6)	1.4 (0.1-6.1)	0.0 (0.1-19.5)	1.7 (0.4-4.4)	6.5 (2.6-12.8)	1.9 (0.5-4.8)	2.1 (0.1-9.0)	2.8 (0.9-6.5)	2.7 (0.2-11.4)
Bocavirus	0.2 (0.0-0.8)	0.0 (0.0-1.9)	0.0 (0.0-5.1)	0.0 (0.0-19.5)	0.6 (0.0-2.5)	0.0 (0.0-3.9)	0.0 (0.0-2.3)	0.0 (0.0-7.4)	0.0 (0.0-2.6)	0.0 (0.0-9.5)
Coronavirus	2.6 (1.5-4.1)	5.7 (3.0-9.5)	2.9 (0.5-8.6)	5.9 (0.3-23.4)	4.6 (2.1-8.4)	8.7 (4.1-15.6)	1.9 (0.5-4.8)	4.2 (0.7-12.3)	0.7 (0.0-3.1)	0.0 (0.0-9.5)

AECOPD, acute exacerbations of chronic obstructive pulmonary disease; CI, confidence interval; HRV, human rhinovirus; N, number of patients per given category; PCR, polymerase chain reaction; RSV, respiratory syncytial virus

Supplementary Table 4. Bacterial and viral load results by quantitative PCR at scheduled stable-state or exacerbation visits.

	Mean load, copies/mL sputum (SD)	
Species	**Scheduled visits (N=554)**	**AECOPD visits (N=209)**
H. influenzae	1.6×10^8 (5.4×10^8)	3.6×10^8 (11.3×10^8)
M. catarrhalis	1.9×10^8 (7.3×10^8)	1.8×10^8 (2.7×10^8)
S. pneumoniae	1.0×10^8 (3.1×10^8)	1.2×10^8 (2.3×10^8)
HRV	3.1×10^6 (13.9×10^6)	7.0×10^6 (18.7×10^6)

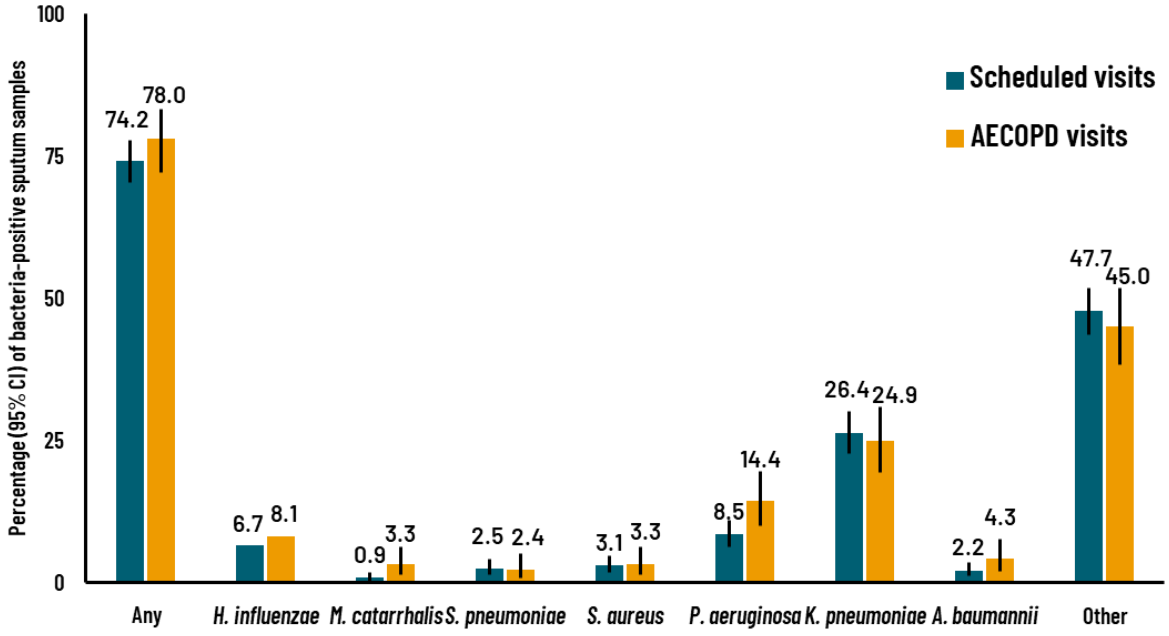
AECOPD, acute exacerbations of chronic obstructive pulmonary disease; HRV, human rhinovirus; N, number of sputum samples per given category; SD, standard deviation

Supplementary Table 5. Prevalence of bacteria (no bacteria or any bacteria) by culture and PCR at scheduled stable-state or exacerbation visits according to antibiotic administration before sputum sample collection.

	Scheduled visits			AECOPD visits		
	Number of sputum samples	**No bacteria, N (%)**	**Any bacteria, N (%)**	**Number of sputum samples**	**No bacteria, N (%)**	**Any bacteria, N (%)**
Culture						
No antibiotic administered	553	142 (25.7)	411 (74.3)	195	41 (21.0)	154 (79.0)
Antibiotic administered	1	1 (100)	0 (0)	14	5 (35.7)	9 (64.3)
PCR						
No antibiotic administered	545	179 (32.8)	366 (67.2)	180	46 (25.6)	134 (74.4)
Antibiotic administered	1	1 (100)	0 (0)	14	3 (21.4)	11 (78.6)

AECOPD, acute exacerbations of chronic obstructive pulmonary disease; N, number of sputum samples per given category

Supplementary Figure 1. Percentage (95% confidence intervals) of sputum samples positive for bacteria by culture analysis, by type of visit: scheduled or unscheduled AECOPD visit**.**



AECOPD, acute exacerbations of chronic obstructive pulmonary disease; CI, confidence interval