

Amoxicillin concentrations in relation to beta-lactamase activity in sputum during exacerbations of chronic obstructive pulmonary disease

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Background: Acute exacerbations of chronic obstructive pulmonary disease (COPD) are often treated with antibiotics. Theoretically, to be maximally effective, the antibiotic concentration at sites of infection should exceed the minimum inhibitory concentration at which 90% of the growth of potential pathogens is inhibited (MIC_{90}). A previous study showed that most hospitalized COPD patients had sputum amoxicillin concentrations $<MIC_{90}$ when treated with amoxicillin/clavulanic acid. Those with adequate sputum concentrations had better clinical outcomes. Low amoxicillin concentrations can be caused by beta-lactamase activity in the lungs. This study investigated whether patients with sputum amoxicillin concentrations $<MIC_{90}$ had higher beta-lactamase activity in sputum than patients with a concentration $\geq MIC_{90}$.

Methods: In total, 23 patients hospitalized for acute exacerbations of COPD and treated with amoxicillin/clavulanic acid were included. Sputum and serum samples were collected at day 3 of treatment to determine beta-lactamase activity in sputum and amoxicillin concentrations in both sputum and serum.

Results: We found no difference in beta-lactamase activity between patients with sputum amoxicillin concentrations $<MIC_{90}$ and $\geq MIC_{90}$ ($P=0.79$). Multivariate logistic regression analysis showed no significant relationship between beta-lactamase activity and sputum amoxicillin concentrations $<MIC_{90}$ or $\geq MIC_{90}$ (odds ratio 0.53; 95% confidence interval 0.23–1.2; $P=0.13$). Amoxicillin concentrations were $<MIC_{90}$ in 78% of sputum samples and in 30% of serum samples.

Conclusion: In patients treated with amoxicillin/clavulanic acid for an acute exacerbation of COPD, sputum beta-lactamase activity did not differ between those with sputum amoxicillin concentrations $<MIC_{90}$ or $\geq MIC_{90}$. The finding that the majority of patients had sputum amoxicillin concentrations $<MIC_{90}$ suggests that current treatment with antibiotics for acute exacerbations of COPD should be optimized.

Keywords: chronic obstructive pulmonary disease, exacerbation, amoxicillin, clavulanic acid, MIC_{90} , concentration

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of chronic morbidity and mortality throughout the world. The prevalence and burden of COPD are projected to increase in the coming decades due to continued exposure to COPD risk factors and the changing age structure of the world's population, with COPD likely to become the third leading cause of death by 2020.¹ Morbidity and mortality among patients with COPD are in large part related to acute exacerbations (AECOPD), which impair respiratory, physical, social, and emotional functioning both acutely and longitudinally.^{2–4}

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The management of AECOPD is empirical and includes oral corticosteroids, often combined with antibiotics, although the need to prescribe these antibiotics is still not convincingly demonstrated.⁵ Most placebo-controlled antibiotics trials that have been performed have important limitations and are difficult to compare because different definitions of COPD and AECOPD and different endpoints have been used.^{6,7} Further, these trials were conducted several decades ago, before systemic steroids were widely introduced for the treatment of AECOPD.^{8–11} In a more recent placebo-controlled study by Llor et al, treatment with amoxicillin/clavulanic acid did show a beneficial effect; however, only 17% of these patients received systemic steroids.¹² In a study by Daniels et al, in which the add-on effect of antibiotics was investigated, no difference in clinical outcome after 30 days was observed.¹³

Theoretically, to be maximally effective, the antibiotic concentration at sites of infection should exceed the minimum inhibitory concentration at which 90% (MIC_{90}) of the growth of potential COPD pathogens such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* is inhibited. Levels of antimicrobial agents in sputum, where as a representation of the site of infection many potential pathogenic microorganisms are located, may be a more relevant predictor of efficacy of antibiotics in treatment of an AECOPD than concentrations in serum.^{14–16} An antibiotic widely used in the treatment of AECOPD is amoxicillin/clavulanic acid.

In a former study by Brusse-Keizer et al, in which 33 COPD patients were treated with amoxicillin/clavulanic acid for AECOPD, sputum amoxicillin concentrations proved to be an important determinant of clinical outcome. Patients with sputum amoxicillin concentrations $<MIC_{90}$ were hospitalized for 4 days longer than patients with an amoxicillin concentration in sputum $\geq MIC_{90}$ (7 versus 11 days). Moreover, 67% of patients in this study had a sputum amoxicillin concentration $<MIC_{90}$.¹⁷

The sputum amoxicillin concentration may differ markedly from the concentrations in serum due to various factors, such as the diffusion of amoxicillin into the airways,¹⁸ which could be both a host-related as well as a drug-related factor.

A well-known drug-related factor associated with amoxicillin is the susceptibility of amoxicillin to breakdown by beta-lactamase enzymes. In COPD, there are several potential pathogens (eg, *H. influenza* and *M. catarrhalis*) that produce these beta-lactamase enzymes.¹⁹ The use of beta-lactamase inhibitors, such as clavulanic acid, allows inactivation of certain beta-lactamases.²⁰ An excessive beta-lactamase activity

in sputum of COPD patients compared with the dosage of clavulanic acid could possibly be an explanation for the earlier observed low numbers of COPD patients in which an amoxicillin concentration above the MIC_{90} was reached.

We therefore conducted a study to investigate whether there was any difference in beta-lactamase activity between COPD patients with a sputum amoxicillin concentration $<MIC_{90}$ and those with a concentration $\geq MIC_{90}$.

Materials and methods

Patients

This study was part of the Cohort of Mortality and Inflammation in COPD (COMIC) study, a single-center prospective cohort study on the immune status of COPD patients as a determinant of survival. From December 2005 until April 2010, 795 patients were included in the cohort, with a follow-up period of 3 years. To be eligible for the cohort, patients had to meet the following criteria: a clinical diagnosis of COPD, as defined by the Global Initiative for Chronic Obstructive Lung Disease criteria;²¹ current or former smoker; age 40 years or over; no medical condition compromising survival within the follow-up period or serious psychiatric morbidity; absence of any other active lung disease (eg, sarcoidosis); no maintenance therapy with antibiotics; and ability to speak Dutch.

In this exploratory study, we had no data available on which we could base our power calculations. We therefore included all patients from the COMIC study when they were hospitalized for an AECOPD that was treated with amoxicillin/clavulanic acid between November 2009 and March 2010.

An AECOPD was defined as an acute event characterized by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medication.^{21,22} Patients with pneumonia were not excluded, and pneumonia was defined as an acute respiratory infection combined with an infiltrate of the lungs, which was visible on an X-ray of the chest. The infiltrate had to be non-pre-existent, nor reasonably caused by another cause than pneumonia. Further, patients had to be able to produce a sputum sample.

The medical ethics committee of Medisch Spectrum Twente, Enschede, the Netherlands approved the COMIC study and the amendment for the current study, and all patients provided written informed consent for both the COMIC study itself and the amendment. All patients received usual care, which included oral corticosteroids and amoxicillin/clavulanic acid started on the day of admission. Amoxicillin/clavulanic acid was administered according to regular care, which was either orally (500/125 mg three or four times a day) or intravenously (1,000/200 mg four

times a day). Some patients received oral and intravenous amoxicillin/clavulanic acid sequentially.

Outcome measures

On the first day of admission, C-reactive protein was measured in blood using the NyoCard® CRP Single Test (Clindia Diagnostics, Leusden, the Netherlands). On the third day of treatment with amoxicillin/clavulanic acid, sputum and serum samples were collected. Sample collection was performed on the third day of treatment because, theoretically, steady-state amoxicillin concentrations in both serum and sputum should be reached by then.²³ Serum samples were stored at -80°C . Sputum samples were stored at -80°C after a culture was performed.

Before measuring amoxicillin concentrations and beta-lactamase activity in sputum, sputum samples were thawed and homogenized using a MagNA lyser (3×60 seconds at 6,500 rpm; Roche Diagnostics, Indianapolis, IN, USA). Lysing of cells during the homogenization process was confirmed by microscopy. After homogenization, vials were centrifuged at 10,000 g for 5 minutes to separate cell debris. Amoxicillin concentrations in serum and homogenized sputum samples were determined using a high-performance liquid chromatography/tandem mass spectrometry method.

For amoxicillin, an a priori cut-off level of 2 mg/L was defined as an adequate concentration in both serum and sputum. This value corresponds to the MIC_{90} for amoxicillin/clavulanic acid. The MIC_{90} used in this study is derived from local susceptibility tests from the Regional Laboratory of Public Health and is comparable with data published by the European Committee On Susceptibility Testing.²⁴

Beta-lactamase activity was measured directly in homogenized sputum samples by measuring the turnover rate of nitrocefin (Calbiochem, San Diego, CA, USA). Nitrocefin is a beta-lactam with chromogenic properties; it changes color from yellow to red under the influence of beta-lactamase activity. Nitrocefin turnover was measured with spectrophotometry at $\lambda=490\text{ nm}$ in a mixture of 50 μL of homogenized sputum and 50 μL of a 0.025% nitrocefin solution. Beta-lactamase activity was quantified by comparing the measured activity with the activity of a beta-lactamase positive lysate from a *H. influenzae* culture. Beta-lactamase activity was calculated as a percentage of the activity of the *H. influenzae* lysate.

Statistical analysis

Continuous variables are expressed as the mean (standard deviation [SD]) or as the median (interquartile range [IQR]).

Categorical variables are displayed as numbers (percentages). The crude relationship between beta-lactamase activity and sputum amoxicillin concentrations ($<\text{MIC}_{90}$ or $\geq\text{MIC}_{90}$) was analyzed using the Mann–Whitney *U* test. To identify confounders in this relationship, first the association of a priori selected possible confounders with beta-lactamase activity was analyzed by Pearson correlation tests, independent samples *t*-tests, and analysis of variance for normally distributed (continuous/dichotomous/categorical) variables. For non-normally distributed variables, this was performed with, respectively, Spearman correlation tests, Mann–Whitney *U* tests, and Kruskal–Wallis tests. Variables associated with beta-lactamase activity with a significance of $P<0.15$ were tested for an association with sputum amoxicillin concentration ($<\text{MIC}_{90}$ or $\geq\text{MIC}_{90}$). For categorical variables, these associations were tested by chi-square tests or by Fisher's Exact tests, and for continuous variables by independent samples *t*-tests or Mann–Whitney *U* tests. Variables that were also associated with sputum amoxicillin concentration with a significance of $P<0.15$ were considered as potential confounders in the relationship between beta-lactamase activity and sputum amoxicillin concentration and were entered in a multivariate logistic regression model. Subsequently, variables with the highest *P*-values were eliminated step by step, until the fit of the model decreased significantly, based on the $-2\log$ likelihood. The statistical analyses were performed using Statistical Package for the Social Sciences version 15.0 software (SPSS Inc., Chicago, IL, USA).

Results

Between November 2009 and March 2010, 147 patients were screened for eligibility (Figure 1). Of the 30 patients included, 23 provided a sufficient amount of sputum. Table 1 shows the demographic and clinical characteristics of these 23 patients. The organisms isolated in all patients were confirmed to be susceptible to amoxicillin.

The univariate analysis showed no difference in beta-lactamase activity between patients with a sputum amoxicillin concentration $<\text{MIC}_{90}$ and patients with a concentration $\geq\text{MIC}_{90}$ with, respectively, a median beta-lactamase activity of 0.35 (IQR 0.26–0.59) and 0.32 (IQR 0.18–0.62; $P=0.79$). Also when individual data of beta-lactamase activity and sputum amoxicillin concentrations were plotted in a scatter diagram (Figure 2) no correlation could be observed ($r=-0.06$, $P=0.80$). In 18 of 23 sputum samples (78%), amoxicillin concentrations were below the MIC_{90} . In six of those samples (26%), the amoxicillin concentration was undetectable. Seven of 23 serum samples (30%)

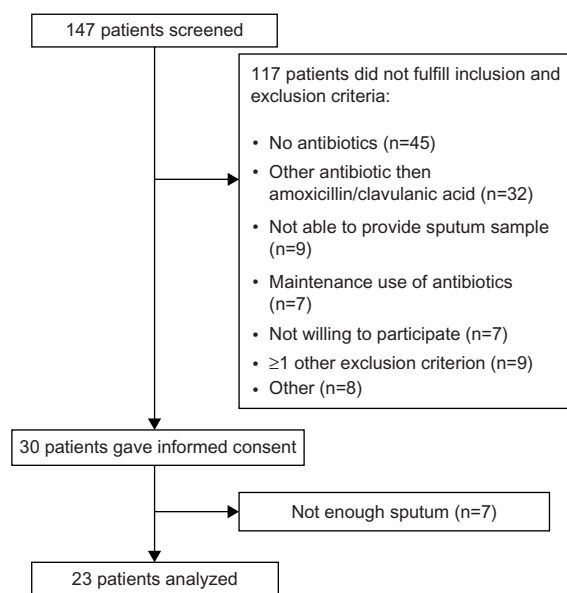


Figure 1 Flowchart of study inclusion.

had an amoxicillin concentration below the MIC₉₀. All serum samples had detectable levels of amoxicillin.

Only route of administration, median daily clavulanic acid dose, and C-reactive protein concentration at admission were univariately associated with beta-lactamase activity ($P < 0.15$; Table 2) and were tested for an association with sputum

Table 1 Baseline patient demographic and clinical characteristics

Characteristic	n=23
Age, years (SD)	71.0 (8.9)
Sex	
Male (%)	17 (74%)
Female (%)	6 (26%)
GOLD classification	
I (%)	2 (9%)
II (%)	6 (26%)
III (%)	8 (35%)
IV (%)	7 (30%)
Route of administration of amoxicillin/clavulanic acid	
Oral (%)	15 (65%)
Intravenous (%)	8 (35%)
Median daily dose of clavulanic acid, mg (IQR)	375 (375–800)
Median concentration of amoxicillin	
In serum, mg/L (IQR)	4.7 (0.72–6.35)
In sputum, mg/L (IQR)	0.6 (0.00–1.74)
Median beta-lactamase activity, as % of reference (IQR)	0.34 (0.26–0.58)
Median CRP concentration at admission, mg/L (IQR)	40 (25–152)
Patients with both exacerbation of COPD and pneumonia	11 (48%)
Simultaneous use of other antibiotics	5 (22%)

Abbreviations: COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; GOLD, Global Initiative for Chronic Obstructive Lung Disease; IQR, interquartile range; SD, standard deviation.

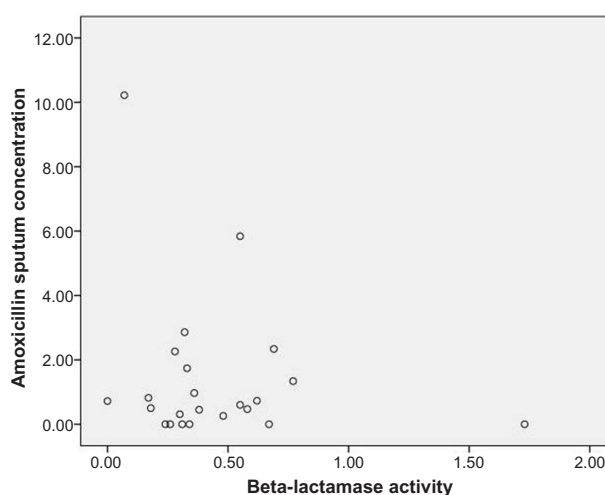


Figure 2 Scatter diagram of amoxicillin sputum concentration and beta-lactamase activity.

Table 2 Potential confounders: association with beta-lactamase activity

	Association with beta-lactamase activity	
	Median beta-lactamase activity (% of reference) (IQR)	P-value
Sex		
Male	0.34 (0.25–0.60)	0.86
Female	0.36 (0.23–0.85)	
Exacerbation with pneumonia		
No	0.33 (0.27–0.46)	0.31
Yes	0.55 (0.18–0.69)	
GOLD classification		
I	0.55 (0.48–0.55)	0.46
II	0.44 (0.27–0.61)	
III	0.30 (0.12–0.50)	
IV	0.36 (0.24–0.67)	
Route of administration		
Oral	0.31 (0.18–0.48)	0.06
Intravenous	0.57 (0.33–0.67)	
Concurrent use of other antibiotics		
No	0.34 (0.24–0.59)	0.77
Yes	0.36 (0.28–0.59)	
Clavulanic acid, daily dose (mg)		
375	0.28 (0.17–0.37)	0.08
500	0.48 (0.33–0.48)	
800	0.57 (0.33–0.67)	
	Correlation with beta-lactamase activity (Spearman's rho)	P-value
Age	–0.05	0.81
Amoxicillin level in serum mg/L	0.22	0.30
CRP concentration at admission mg/L	–0.37	0.09

Abbreviations: CRP, C-reactive protein; GOLD, Global Initiative for Chronic Obstructive Lung Disease; IQR, interquartile range.

amoxicillin concentration. All three were also significantly associated with sputum amoxicillin concentration $< \text{MIC}_{90}$ or $\geq \text{MIC}_{90}$ (Table 3). Multivariate logistic regression analysis showed no significant relationship between beta-lactamase activity and sputum amoxicillin concentrations $< \text{MIC}_{90}$ versus $\geq \text{MIC}_{90}$ (odds ratio 0.53 for a 0.1% increase in beta-lactamase activity; 95% confidence interval 0.23–1.2; $P=0.13$). The likelihood of reaching a sputum concentration $\geq \text{MIC}_{90}$ was 80 times greater when amoxicillin/clavulanic acid was administered intravenously versus via the oral route (odds ratio 80.6; 95% confidence interval 1.6–4100; $P=0.03$).

Discussion

No difference in beta-lactamase activity was found between patients with an amoxicillin concentration in sputum $< \text{MIC}_{90}$ and patients with a concentration $\geq \text{MIC}_{90}$ when treated with amoxicillin/clavulanic acid. Although we found that 78% of the patients had a low sputum concentration of amoxicillin ($< \text{MIC}_{90}$), beta-lactamase activity does not seem to be the reason for this observation.

Beta-lactamase activity could not explain the low numbers of patients with an adequate amoxicillin concentration in sputum, so it seems that there should be other host-related or drug-related factors that influence the penetration across the blood-bronchus and alveolar-capillary barriers.

The most important host-related factor is the integrity of the anatomical barriers which may be damaged by inflammation and mechanical injury. In the presence of inflammation, the distribution of amoxicillin may be altered because of increased membrane permeability.^{25,26} As observed in this and our previous study a high level of C-reactive protein, a marker of systemic inflammation, was related to higher amoxicillin levels.¹⁷ C-reactive protein levels could

therefore possibly be used as a marker to determine COPD patients in whom adequate concentrations could be reached. However, this does not solve the problem of low concentrations in the majority of patients; this is a worrying feature, especially because our previous study showed that this is also associated with significantly worse clinical outcomes.¹⁷ This warrants further investigation into more optimal treatment in these patients.

Since beta-lactam antibiotics such as amoxicillin do not cross membranes readily,²⁷ it might be interesting to look at other antibiotics for the treatment of AECOPD that have a better penetration in sputum.²⁸ Also, the possibility of individually tailored amoxicillin dosing could be investigated, since it has been shown that increased systemic dosing of amoxicillin is associated with increased levels in sputum. We observed that intravenous administration was associated with a higher probability of reaching a sputum amoxicillin concentration $\geq \text{MIC}_{90}$ and led to higher serum concentrations (data not shown), but still 50% of patients had a concentration $< \text{MIC}_{90}$. Further, due to the wide confidence interval that was observed with this probability and since we did not observe any differences in the numbers of patients who reached the MIC_{90} between intravenous and oral administration in our earlier study,¹⁷ we cannot recommend a preference for use of either intravenous or oral administration to reach adequate amoxicillin sputum levels.

Alternative routes of administration such as inhalation of antibiotics could be considered. Aerosolized antibiotics have been proven to deliver high concentrations of antibiotics into the airways with low systemic bioavailability. Stockley et al treated patients with bronchiectasis with nebulized amoxicillin and observed significant reductions in sputum purulence and volume after failure with the same drug given orally.²⁹ It would therefore be interesting to administer amoxicillin/clavulanic acid via inhalation to reach the MIC_{90} , and perhaps to improve the efficacy of antibiotic treatment, as seen in cystic fibrosis.^{29,30}

The observed concentration of amoxicillin in serum is in the range of concentrations found in earlier published studies, which showed concentrations between 3.87 and 45.2 mg/L. Observed concentrations of amoxicillin in sputum ranged between 0.23 and 4.4 mg/L.^{31–36} However, these concentrations were measured in non-COPD patients and in healthy subjects.

Our study could have been influenced by selection bias, since we only included patients who were able to produce a sufficient amount of sputum on day 3. There might have been

Table 3 Potential confounders: association with $< \text{MIC}_{90}$ or $\geq \text{MIC}_{90}$

	Association with $< \text{MIC}_{90}$ or $\geq \text{MIC}_{90}$ in sputum (n=23)		P-value
	$< \text{MIC}_{90}$ in sputum	$\geq \text{MIC}_{90}$ in sputum	
Route of administration			
Oral, n (%)	14 (93)	1 (7)	0.03
Intravenous, n (%)	4 (50)	4 (50)	
Median daily clavulanic acid dose, mg (IQR)	375 (375–575)	800 (589–800)	0.04
Median CRP concentration at admission, mg/L (IQR)	38 (21–57)	197 (56–206)	0.06

Abbreviations: CRP, C-reactive protein; IQR, interquartile range; MIC, minimum inhibitory concentration.

a difference in beta-lactamase activity and/or amoxicillin concentration between patients who were able to produce sputum and those who were not. Patients who were able to produce sputum may have had other characteristics in terms of presence/absence of bacteria or cause of exacerbation (eg, viral/bacterial infection). To overcome this issue, induction of sputum with hypertonic saline and the effects on possible dilution of amoxicillin concentration could be explored. Sputum induction has proven to be safe even during exacerbations in COPD patients.³⁷

Since the majority of patients had an amoxicillin concentration $< \text{MIC}_{90}$, the beta-lactamase activity of these patients could be compared to the beta-lactamase activity of only a small group of patients with a concentration $\geq \text{MIC}_{90}$. Although there seems to be no difference in median beta-lactamase activity, this study cannot definitely conclude that no relationship exists, and further studies are still necessary.

Conclusion

We observed no relationship between beta-lactamase activity and sputum amoxicillin concentration ($< \text{MIC}_{90}$ or $\geq \text{MIC}_{90}$) in patients treated with amoxicillin/clavulanic acid for an acute exacerbation in COPD. More studies are necessary to confirm this finding. Further, we repeatedly observed that the majority of patients had low sputum concentrations of amoxicillin ($< \text{MIC}_{90}$), suggesting that current treatment with antibiotics should be optimized.

Disclosure

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