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Etiologic Diagnosis of Adult Bacterial Pneumonia by Culture and PCR Applied to Respiratory Tract Samples

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Respiratory culture and multiplex PCR for *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae* were applied to sputum, nasopharyngeal swabs, and nasopharyngeal aspirates from 235 adult patients with community-acquired pneumonia and 113 controls. Both culture and multiplex PCR performed well with the different samples and appear to be useful as diagnostic tools.

As an etiologic agent can rarely be identified in more than 50% of patients with community-acquired pneumonia (CAP) (9), further development of diagnostic methods has been encouraged (4, 10).

The aim of the present study was to estimate the diagnostic accuracy of respiratory culture and a single-run multiplex PCR (mPCR) for specific genes of *Streptococcus pneumoniae* (*lytA*), *Haemophilus influenzae* (16S rRNA, with verification by PCR for *P6*), *Mycoplasma pneumoniae* (*P1*), and *Chlamydophila pneumoniae* (*ompA*) (12) applied to respiratory samples in CAP patients.

(The study was approved by the ethics committee of the Örebro County Council [868-1999; 556-2000].)

In a prospective study described previously (14), 235 hospitalized CAP patients with X-ray infiltrates were enrolled. Their median age was 71 years (age range, 18 to 96 years), 40% belonged to severity risk classes IV and V, 14% had chronic obstructive pulmonary disease, and 22% were smokers. In 82% of the patients, the interval from the onset of illness to admission was 0 to 7 days.

During the study period, 113 adult controls (median age, 69 years) without respiratory symptoms were enrolled. They were hospitalized for skin infection ($n = 14$), urinary tract infection ($n = 14$), arthritis or spondylitis ($n = 6$), or planned orthopedic or urological surgery ($n = 79$).

The results of the respiratory cultures and mPCR analyses performed with specimens from the patients and the controls are shown in Table 1. Sputum samples were analyzed if there were more than five leukocytes per squamous epithelial cell (6). To obtain a nasopharyngeal aspirate (NpA), secretions from the nasopharynx were aspirated by a catheter connected to an electronic suction device. About 1 ml NaCl (0.85%) was then aspirated to collect the secretions situated within the catheter. Culture and mPCR of sputum, nasopharyngeal swabs (NpSs), and NpAs were performed as described previously (12). Multiplex PCR was performed blindly with samples previously frozen at −70°C.

To establish the definite etiologies of CAP, we performed the following: blood culture for 235 patients, the NOW *S. pneumoniae* urinary antigen test (Binax) for 215 patients, the complement fixation test for *M. pneumoniae* (8) with paired serum specimens for 216 patients, the microimmunofluorescence test for *C. pneumoniae* (3) with paired serum specimens for 216 patients, and the indirect immunofluorescence test for *H. influenzae* (13) with paired serum specimens for 48 patients.

Our criteria for definite CAP etiologies were as follows: for *S. pneumoniae*, a positive blood culture or a positive urinary antigen test (only visible result lines at least as intense as the control line were considered positive; other results were considered negative) (14); for *H. influenzae*, a positive blood culture or a positive indirect immunofluorescence test, i.e., a fourfold or greater rise in the immunoglobulin G (IgG) antibody titer against the patient's own *H. influenzae* isolate (13); for *M. pneumoniae*, a positive complement fixation test result, i.e., a fourfold or greater rise in the antibody titer; and for *C. pneumoniae*, a positive microimmunofluorescence test result, i.e., a fourfold or greater rise in the IgG antibody titer or an IgM titer ≥1/16.

The pathogens for which tests were performed were established as the definite etiologic agents in 79 (34%) of the 235 CAP patients, i.e., *S. pneumoniae* in 39 patients (17%), *H. influenzae* in 23 patients (9.8%), *M. pneumoniae* in 13 patients (5.5%), *C. pneumoniae* in 2 patients (0.9%), both *S. pneumoniae* and *H. influenzae* in 1 patient (0.4%), and both *H. influenzae* and *C. pneumoniae* in 1 patient (0.4%).

Tables 2 and 3 show the sensitivities and the specificities of the respiratory cultures and mPCR analyses.

In the specificity calculations, our criteria for the exclusion of pathogens as etiologic agents were as follows: for *S. pneumoniae*, a negative blood culture plus a negative urinary antigen test result; for *M. pneumoniae*, a negative complement fixation test result with paired serum specimens; and for *C. pneumoniae*, a negative microimmunofluorescence test result with paired serum specimens. No diagnostic method was available to rule out *H. influenzae* as the etiologic agent, as the indirect immunofluorescence test could be performed only for culture-positive patients. Thus, no specificity could be calculated. However, among the 54 CAP patients with definite etiologies other than *H. influenzae*, this pathogen was identified by the culture of sputum for 0% of the patients (0 of 28), NpSs

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TABLE 1. Analyses of respiratory tract secretions for *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae* in 235 patients with CAP and 113 controls

Test	CAP patients					Controls				
	No. of samples with which test was performed	No. of samples with positive results for[a]:				No. of samples with which test was performed	No. of samples with positive results for[a]:			
		S. pneumoniae	*H. influenzae*	*M. pneumoniae*	*C. pneumoniae*		*S. pneumoniae*	*H. influenzae*	*M. pneumoniae*	*C. pneumoniae*
Sputum culture	112	36	21			0				
Sputum mPCR	103	55	24	14	1	0				
Nasopharyngeal swab culture	233	46	34			113	2	2		
Nasopharyngeal swab mPCR	194	35	20	14	1	110	3	2	0	0
Nasopharyngeal aspirate culture	220	63	49			113	5	2		
Nasopharyngeal aspirate mPCR	218	79	53	17	3	110	9	5	1	0

[a] An empty space indicates that the analysis was not performed.

for 3.8% of the patients (2 of 53), and NpAs for 4.3% of the patients (2 of 47) and by mPCR of sputum for 7.7% of the patients (2 of 26), NpSs for 4.3% of the patients (2 of 47), and NpAs for 8.5% of the patients (4 of 47). These results indicate that the tests for *H. influenzae* had high specificities.

The usefulness of respiratory culture and mPCR was further supported by the low frequencies of pathogens identified in the control group (Table 1).

Sputum remains the respiratory sample of choice for establishment of the etiology of CAP, as it is well studied for the identification of both typical (1) and atypical (2, 7, 11) bacteria. However, the present study indicates that when sputum is unobtainable, NpSs or NpAs could be used for diagnostic testing. NpAs appear to be more useful than NpSs for establishment of the etiology of CAP, as NpAs generally showed higher sensitivities and as NpAs and sputum samples performed similarly. Among 99 patients for whom both sputum samples and NpAs were analyzed, the two samples gave identical results for *S. pneumoniae* for 88% of the patients ($n = 87$) by culture and for 85% of the patients ($n = 84$) by mPCR, and the two samples gave identical results for *H. influenzae* for 92% of the patients ($n = 91$) by culture and for 86% of the patients ($n = 85$) by mPCR. However, NpSs displayed higher specificities than NpAs for *S. pneumoniae*, and NpS culture has previously been found to be specific for pneumococcal pneumonia (5). Thus, if a high specificity for *S. pneumoniae* is mainly sought, NpSs should reasonably be preferable to NpAs.

In patients not treated with antibiotics, culture and mPCR had similar yields for *S. pneumoniae* and *H. influenzae* (Table 2). Antibiotics were taken prior to the collection of one or more respiratory samples in 66 CAP patients. In total, 138 respiratory samples were collected during antibiotic treatment, and there was no difference between the results of culture and mPCR for the identification of *H. influenzae*, while *S. pneumoniae* was identified by culture in 4.3% of the patients ($n = 6$) and by mPCR in 14% of the patients ($n = 20$) ($P = 0.004$, chi-square test). During antibiotic treatment in a previous study (15), PCR for *S. pneumoniae* with sputum often remained positive, while sputum culture became negative. Another major advantage of mPCR compared with culture in antibiotic-treated patients is its ability to identify *M. pneumoniae* and *C. pneumoniae*, as an atypical etiology may be a reason for a nonresponse to first-line CAP treatment.

Since the four pathogens identified by mPCR are common etiologic agents of CAP (9) and since the sensitivities of mPCR were moderate to high, negative mPCR results may be used to

TABLE 2. Performance of culture and mPCR for *Streptococcus pneumoniae* and *Haemophilus influenzae* for pneumonia patients without ongoing antibiotic treatment

Test	*S. pneumoniae*		Sensitivity[a] for *H. influenzae*
	Sensitivity[a]	Specificity[b]	
Sputum culture	86 (12/14)	66 (42/64)	89 (8/9)
Sputum mPCR	92 (12/13)	42 (24/57)	78 (7/9)
Nasopharyngeal swab culture	58 (19/33)	82 (107/130)	85 (17/20)
Nasopharyngeal swab mPCR	61 (19/31)	87 (89/102)	80 (12/15)
Nasopharyngeal aspirate culture	85 (23/27)	74 (89/121)	100 (20/20)
Nasopharyngeal aspirate mPCR	93 (25/27)	66 (79/119)	85 (17/20)

[a] Reported as percent (number of positive samples/number of samples with the definite etiology).
[b] Reported as percent (number of negative samples/number of samples without the etiology).

TABLE 3. Performance of mPCR for *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* in pneumonia patients without ongoing treatment with fluoroquinolones, macrolides, or tetracyclines

Sample type	*M. pneumoniae*		*C. pneumoniae*	
	Sensitivity[a]	Specificity[b]	Sensitivity[a]	Specificity[b]
Sputum	88 (7/8)	93 (81/87)	100 (1/1)	100 (94/94)
Nasopharyngeal swab	67 (6/9)	96 (151/158)	100 (1/1)	100 (166/166)
Nasopharyngeal aspirate	90 (9/10)	97 (175/180)	100 (3/3)	100 (187/187)

[a] Reported as percent (number of positive samples/number of samples with the definite etiology).
[b] Reported as percent (number of negative samples/number of samples without the etiology).

rule out these etiologies. When mPCR is positive for a single pathogen, the negative results for the other three pathogens increase the likelihood that the positive result is truly positive.

Based on the present results, we suggest that respiratory culture and mPCR applied to sputum, NpSs, and NpAs can be used to obtain presumptive diagnoses of the etiology of CAP in adult populations with expectedly low rates of carriage of respiratory pathogens. These presumptive diagnoses can guide antibiotic therapy and support treatment with narrow-spectrum antibiotics.

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