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Comparison of rapid immunochromatographic assays using sputum and urine for *Streptococcus pneumoniae* detection in adult patients with respiratory tract infection

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Aim: *Streptococcus pneumoniae* is the most frequently detected bacterium in pneumonia. RAPIRUN *Streptococcus pneumoniae* (RAPIRUN) using sputum and BinaxNow *Streptococcus pneumoniae* (BinaxNow) using urine have been used as rapid diagnostic methods for *S. pneumoniae* detection in Japan; however, their correlation with quantitative culture tests has not been well evaluated.

Methods: A prospective study was conducted on adult patients with respiratory tract infections whose sputum and urine samples were available in six hospitals. Sputum and urine samples were tested at each site, and quantitative sputum cultures were performed. The performance of RAPIRUN and BinaxNow was compared in cases in which quantitative culture showed *S. pneumoniae*.

Results: A total of 192 patients were analyzed. Of these, 167 were diagnosed with pneumonia (87.0%) including 161 of community-acquired pneumonia. Of the 192 cases, 86 (44.8%) were culture-proven for *S. pneumoniae*. There were 83 and 57 RAPIRUN- and BinaxNow-positive cases, respectively. The sensitivity and specificity of RAPIRUN were 84.9% and 90.6%, respectively, and those of BinaxNOW were 55.8% and 91.5%, respectively, indicating that RAPIRUN was significantly superior in sensitivity ($p < 0.0001$) with almost equal specificity ($p = 0.317$). Positive and negative percent agreements of both tests were 59.3% (κ, 0.114 [95% CI, 0.053–0.281]) and 99.1% (κ, 0.942 [95% CI, 0.830–1]), respectively, indicating they were well matched in specificity but not in sensitivity. The positivity rate of RAPIRUN increased with an increase in the number of bacteria ($p< 0.0001$) but not BinaxNow ($p = 0.275$).

Conclusion: In adult patients with respiratory tract infections in whom sputum collection is feasible, RAPIRUN will increase the diagnostic efficacy of *S. pneumoniae* infection.

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Key words: RAPIRUN, BinaxNow, *Streptococcus pneumoniae*, quantitative culture tests

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Introduction

Pneumonia is the fifth leading cause of death in Japan[1]. *Streptococcus pneumoniae* is the most common causative organism of community-acquired pneumonia (CAP) in adults, and 20−25% of CAP cases are thought to be caused by this organism[2-4]. Invasive pneumococcal diseases (IPD) complicated by bacteremia and meningitis, as well as pneumonia due to *S. pneumoniae* infection, have a high mortality rate of 10−15%[5]. Therefore, early diagnosis of respiratory infections is crucial for early treatment, and accurate diagnosis is essential for better outcomes. Previous studies have shown that the causative organism of CAP is revealed in less than 50% of cases, and in fact, pneumococcal pneumonia may be included among pneumonia cases of unknown etiology. Therefore, the prevalence rate of true pneumococcal pneumonia may be 20−25% or higher[6]. Additionally, the positivity rate of blood culture tests for pneumococcal pneumonia at the onset of IPD is also not high, at 20−25%[7]. This low diagnostic rate can be attributed to the use of rapid diagnostic methods. Gram staining of sputum is a rapid diagnostic method for bacterial pneumonia; however, its usefulness is controversial. First, it is essential to acquire good quality sputum; if antimicrobial agents have already been administered, this must also be considered during determination. Furthermore, the skill of the examiner may also have affected the results. A recent meta-analysis reported that the diagnostic sensitivity of Gram staining for pneumococcal pneumonia was 69%, and specificity was 91% only when good-quality sputum can be collected[8].

The urinary antigen test, BinaxNow *Streptococcus pneumoniae* (BinaxNow, Alere Scarborough, Scarborough, USA), which detects cell wall antigens secreted in urine using an immuno-chromatographic method to separate the capsular polysaccharide of *S. pneumoniae*, is a rapid, simple, and frequently used diagnostic method except in patients with anuria. A meta-analysis by Horita et al. reported a sensitivity of 75% and specificity of 95% in adult pneumococcal infections[9], however, caution is required in determining its usefulness for detecting past pneumococcal infection or colonization[10, 11].

In contrast, RAPIRUN *Streptococcus pneumoniae* (RAPIRUN, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) is a rapid immunochromatographic method for detecting cell wall polysaccharide of *S. pneumoniae*, in the sputum of patients with respiratory tract infections[12, 13]. This method was only approved in Japan in December 2010. The performance of RAPIRUN and BinaxNow was compared in cases where *S. pneumoniae* was found to be positive in culture.

RAPIRUN has a sensitivity of 90.0−94.4% and specificity of 61.1−95.7%, and those of BinaxNow are 53.7−62.0% and 82.4−96.7%, respectively, indicating that RAPIRUN has higher sensitivity and specificity than BinaxNow[12-15]. However, no report in the existing literature evaluates the correlation between the positivity rate and the amount of *S. pneumoniae* in sputum by quantitative culture tests. This prospective study compared the performance of RAPIRUN and BinaxNow in approximately 200 adult patients with lower respiratory tract infections in clinical settings after the launch of RAPIRUN in 2010. One of the most distinguished highlights from previous reports is the real-world analysis of the detectable amount of *S. pneumoniae* by RAPIRUN using quantitative sputum culture.

Material and methods

Study design and population

This prospective study was conducted at six medical institutions in Nagasaki Prefecture between September 2011 and May 2015. The study protocol was approved by the Institutional Review Board of Nagasaki University, Japan (approval number: 11032831) and registered on the UMIN website (UMIN000006104). Each medical institutional review board approved this study, and written informed consent was obtained from each patient. All adult inpatients or outpatients (age ≥ 18 years) with signs of lower respiratory infections, including pneumonia, who had undergone sputum exploration were eligible for inclusion in this study. Patients with a provisional diagnosis of respiratory infection were assessed by each investigator to confirm the diagnosis. Patients who could not produce sputum or had anuria were excluded. Patients treated with antibiotics within two weeks prior to diagnosis or taking a low dose of macrolides for an extended period were also excluded. Patients with pneumonia were diagnosed, and their severity was recorded according to the Guidelines for the Management of CAP[16] and hospital-acquired pneumonia (HAP)[17]. The A-DROP and I-ROAD systems were used for severity ratings in CAP and HAP, respectively[16, 17]. All clinical information, including age, sex, underlying diseases, the number of days from onset of symptoms, and the day the tests were performed, were recorded when the patients were registered.

Sample collection and microbiological investigations

Single expectorated sputum and urine samples were collected from patients who provided written informed consent to participate in this study, either during a hospital visit or during the presumptive diagnosis of respiratory infection. Sputum and urine samples were immediately tested using RAPIRUN

and BinaxNOW, respectively, at the institutional laboratory. All sputum samples were transferred to the microbiology laboratory at Chuken Co. Ltd. Nagasaki, Japan, for evaluation of quality, Gram staining, identification, and quantitative culture testing. The quality of the sputum specimen was evaluated using the Miller & Jones criteria based on the appearance of the sputum[18]. Gram staining was performed immediately after the arrival of the samples at the laboratory. The semi-quantitative scoring of Gram staining was based on the number of bacteria per 1,000 oil immersion fields: few = less than one bacterium per field, 1+ = 1–5 bacteria per field, 2+ = 6–30 bacteria per field, and 3+ = more than 30 bacteria per field. The quality of sputum specimens was also evaluated using the Geckler classification at a magnification of ×100, and findings were recorded at a magnification of ×1,000[19]. The sputum samples were cultured at 37˚C for 24 h on blood and chocolate agar. Presumptive colonies of bacteria were picked and identified by biochemical testing (BD BBL CrystalTM GP, Becton and Dickinson). Quantitative culture tests were then performed.

Measurement and interpretation of results obtained with RAPIRUN and BinaxNow

Both tests were performed following the manufacturer's instructions at each participating institution. Briefly, sputum samples collected from swabs in containers were shaken in tubes containing the sample extract and left for 5 min. Then, swab tips were removed from the tubes while squeezing, a filter was placed onto the tube, and the extract was dropped onto the RAPIRUN instrument. The level of urinary antigens for *S. pneumoniae* was measured using BinaxNOW, and the results were interpreted at each institution following the manufacturer's instructions.

Statistics

The sensitivity, specificity, and percent agreement between the two tests were determined using the culture method as the standard. When appropriate, variables were compared using McNemar's test, Wilcoxon rank-sum test, chi-square test, Fisher's exact test, and exact Cochran-Armitage trend test. We calculated the degree of positive and negative agreement between RAPIRUN and BinaxNow, and their respective κ coefficient with 95% confidence intervals. The κ statistic is frequently used to test interrater reliability as well as to evaluate the concordance between tests. Receiver operating characteristic (ROC) analysis was applied to evaluate the performance of RAPIRUN and BinaxNow. We calculated the true-positive rate (TRP, Sensitivity) and false-positive rate (FRP, 1-specificity) at each bacterial volume level from 10^3 to 10^8 CFU/mL. Then, we depicted the ROC curve to plot TPR on Y-axis and FPR on X-axis for the varying value of each threshold from 10^3 to 10^8 CFU/mL levels. Statistical significance was assumed at a p-value <0.05. Analyses were performed with SAS software, version 9.4 (SAS Institute), and JMP 16 Pro software (JMP).

Results

Patient background

Total of 196 cases were recruited in this study and four of them were excluded from analysis due to lack of BinaxNow data. The characteristics of the enrolled patients are presented in Table 1. Of the 192 cases, 167 were diagnosed with pneumonia (87.0%), of which 161 were CAP and six were HAP.

The average age was 70.0 years, and the male-to-female ratio was 124:68. Chronic respiratory disease (52.6%) was the most common underlying disease, followed by cardiac diseases (14.1%). Diabetes mellitus (9.4%) and malignant neoplasms (11.5%) were present at approximately the same degree. Chronic respiratory diseases consisted of bronchial asthma (20.8%), chronic obstructive pulmonary disease (COPD) (16.7%), chronic respiratory failure (6.3%), interstitial pneumonia (6.3%), bronchiectasis (2.6%), chronic bronchitis (2.1%), and sinobronchial syndrome (2.1%). A total of 147 patients (76.6%) had sputum of P1 or higher according to the Miller & Jones classification, and 130 (67.7%) had Geckler quality of 4 or higher according to the Gram stain classification.

Of the 192 cases, 86 (44.8%) were culture-proven for *S. pneumoniae.* Of these, the number of *S. pneumoniae* was more than 10^6 and 10^7 CFU/mL among 70 (81.4%) and 55 (64.0%) patients, respectively. Bacteria such as *Hemophilus influenzae, H. parainfluenzae, Moraxella catarrhalis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli* as possible causative organisms with relatively high bacterial load, were isolated from 24 (12.5%), 23 (12.0%), 13 (6.8%), 12 (6.3%), 5 (2.6%), 4 (2.1%) and 3 (1.6%) cases, respectively. Among the *S. pneumoniae* culture-positive and culture-negative cases, those with underlying diseases and chronic respiratory diseases were significantly more likely to be culture-negative ($p < 0.001$). In total of 67 of *S. pneumoniae* culture-negative with chronic respiratory underlying cases, *H. influenzae, H. parainfluenzae, M. catarrhalis, Staphylococcus* species and others were isolated from 18 (26.9%), 15 (22.4%), 10 (14.9%), 6 (9.0%) and 14 (20.9%) cases, respectively. No bacterial pathogens were isolated in four of these cases. There were no significant

Table 1. Characteristics of the 192 patients with respiratory infections

		Culture of *Streptcoccus pneumoniae*			RAPIRUN			BinaxNow		
	Total (n=192)	Positive (n=86)	Negative (n=106)	*P*	Positive (n=83)	Negative (n=109)	*P*	Positive (n=57)	Negative (n=135)	*P*
Age; mean ± SD	70.0 ± 14.5	68.9 ± 14.3	70.9 ± 14.6	0.269	69.4 ± 14.6	70.7 ± 14.4	0.594	69.3 ± 13.9	70.3 ± 14.7	0.495
Sex (male/female)	124/68	52/34	72/34	0.293	50/33	74/35	0.290	36/21	88/47	0.869
Respiratory diseases										
Pneumonia [CAP:HAP]; n (%)	167 [161:6] (87.0)	77 [74:3] (89.5)	90 [87:3] (84.9)	0.394	75 [72:3] (90.4)	92 [89:3] (84.4)	0.281	53 [51:2] (93.0)	14 [110:4] (84.4)	0.158
Secondary infection or exacerbation of chronic respiratory diseases; n (%)	10 (5.2)	2 (2.3)	8 (7.5)	0.189	3 (3.6)	7 (6.4)	0.519	1 (1.8)	9 (6.7)	0.286
Acute bronchitis; n (%)	11 (5.7)	5 (5.8)	6 (5.7)	1.000	4 (4.8)	7 (6.4)	0.760	3 (5.3)	8 (5.9)	1.000
Others; n (%)	4 (2.1)	2 (2.3)	2 (1.9)	1.000	1 (1.2)	3 (2.8)	0.635	0 (0.0)	4 (3.0)	0.320
Underlying diseases	149 (77.6)	57 (66.3)	92 (86.8)	0.001	57 (68.7)	92 (84.4)	0.014	37 (64.9)	112 (83.0)	0.008
Neoplastic diseases; n (%)	22 (11.5)	9 (10.5)	13 (12.3)	0.821	11 (13.3)	11 (10.1)	0.503	9 (15.8)	13 (9.6)	0.225
Chronic pulmonary diseases; n (%)	101 (52.6)	34 (39.5)	67 (63.2)	0.001	34 (41.0)	67 (61.5)	0.006	20 (35.1)	81 (60.0)	0.002
Cerebrovascular diseases; n (%)	7 (3.6)	4 (4.7)	3 (2.8)	0.702	4 (4.8)	3 (2.8)	0.468	2 (3.5)	5 (3.7)	1.000
Cardiac diseases; n (%)	27 (14.1)	12 (14.0)	15 (14.2)	1.000	12 (14.5)	15 (13.8)	1.000	8 (14.0)	19 (14.1)	1.000
Digestive diseases; n (%)	5 (2.6)	1 (1.2)	4 (3.8)	0.382	1 (1.2)	4 (3.7)	0.392	1 (1.8)	4 (3.0)	1.000
Renal diseases; n (%)	7 (3.6)	4 (4.7)	3 (2.8)	0.702	4 (4.8)	3 (2.8)	0.468	1 (1.8)	6 (4.4)	0.676
Diabetes mellitus; n (%)	18 (9.4)	7 (8.1)	11 (10.4)	0.629	7 (8.4)	11 (10.1)	0.805	7 (12.3)	11 (8.1)	0.419
Collagen vascular diseases; n (%)	8 (4.2)	1 (1.2)	7 (6.6)	0.076	0 (0.0)	8 (7.3)	0.011	0 (0.0)	8 (5.9)	0.108
Others; n (%)	14 (7.3)	5 (5.8)	9 (8.5)	0.582	4 (4.8)	10 (9.2)	0.279	2 (3.5)	12 (8.9)	0.238
Geckler classification										
1; n (%)	5 (2.6)	2 (2.3)	3 (2.8)	0.911	4 (4.8)	1 (0.9)	0.593	3 (5.3)	2 (1.5)	0.428
2; n (%)	5 (2.6)	1 (1.2)	4 (3.8)	-	2 (2.4)	3 (2.8)	-	1 (1.8)	4 (3.0)	-
3; n (%)	52 (27.1)	24 (27.9)	28 (26.4)	-	24 (28.9)	28 (25.7)	-	16 (28.1)	36 (26.7)	-
4; n (%)	55 (28.6)	26 (30.2)	29 (27.4)	-	24 (28.9)	31 (28.4)	-	19 (33.3)	36 (26.7)	-
5; n (%)	73 (38.0)	32 (37.2)	41 (38.7)	-	28 (33.7)	45 (41.3)	-	17 (29.8)	56 (41.5)	-
6; n (%)	2 (1.0)	1 (1.2)	1 (0.9)	-	1 (1.2)	1 (0.9)	-	1 (1.8)	1 (0.7)	-
Miller & Jones classification										
M1; n (%)	3 (1.6)	0 (0.0)	3 (2.8)	0.142	1 (1.2)	2 (1.8)	0.646	0 (0.0)	3 (2.2)	0.188
M2; n (%)	42 (21.9)	17 (19.8)	25 (23.6)	-	15 (18.1)	27 (24.8)	-	14 (24.6)	28 (20.7)	-
P1; n (%)	42 (21.9)	22 (25.6)	20 (18.9)	-	21 (25.3)	21 (19.3)	-	14 (24.6)	28 (20.7)	-
P2; n (%)	48 (25.0)	26 (30.2)	22 (20.8)	-	23 (27.7)	25 (22.9)	-	18 (31.6)	30 (22.2)	-
P3; n (%)	57 (29.7)	21 (24.4)	36 (34.0)	-	23 (27.7)	34 (31.2)	-	11 (19.3)	46 (34.1)	-

differences in age, sex, type of respiratory infection, or sputum quality according to both the Geckler and Miller & Jones classification. Gram stain findings of sputum from 86 pneumococcal culture-positive cases showed gram-positive cocci in all cases, with 1+, 2+, and 3+ in 7 (8.1%), 31 (36.0%), and 48 (55.8%) cases, respectively, of which 41 (47.7%) had phagocytic findings.

There were 83 and 57 RAPIRUN- and BinaxNow-positive cases, respectively. There were no significant differences in age, sex, type of respiratory infections, or sputum quality, including the Geckler and Miller & Jones classification, between the RAPIRUN-positive and -negative arms; however, the positivity rate was significantly lower in patients with underlying diseases ($p < 0.05$), chronic respiratory diseases ($p < 0.01$), and collagen vascular diseases ($p < 0.05$). There were no significant differences in background factors except the underlying disease profile between the BinaxNow positive and negative arms. The positivity rate was significantly lower ($p < 0.01$) in patients with underlying diseases, especially those with chronic respiratory diseases ($p < 0.01$), similar to that of RAPIRUN.

The analysis of 161 CAP cases, including 49 mild, 89 moderate, 21 severe, and 2 critical cases according to the A-DROP scoring system, indicated no significant differences between the pneumococcal culture-positive and -negative arms, RAPIRUN-positive and -negative arms, and BinaxNow-positive and -negative arms in terms of age, sex, severity of CAP, and quality of sputum (data not shown). However, the positivity rate was significantly lower ($p < 0.01$) in patients with underlying diseases, especially those with chronic respiratory diseases ($p < 0.01$) in all groups.

Performance of RAPIRUN and BinaxNow among all 192 cases using the culture-positive method as the standard

Table 2 shows the performances of RAPIRUN and BinaxNow. The sensitivity and specificity of RAPIRUN were 84.9% and 90.6%, respectively, and those of BinaxNOW were 55.8% and 91.5%, respectively, indicating that RAPIRUN was significantly superior in sensitivity ($p < 0.0001$) with almost equal specificity ($p = 0.317$). Positive and negative percent agreements were 59.3% (κ, 0.114 [95% CI, 0.053–0.281]) and 99.1% (κ, 0.942 [95% CI, 0.830–1]), respectively, indicating they were well matched in specificity but not in sensitivity.

Table 2. Performance of RAPIRUN and BinaxNow among all 192 cases using the culture-positive method as the standard

	RAPIRUN	BinaxNow
Sensitivity	84.9 (%)	55.8 (%)
Specificity	90.6 (%)	91.5 (%)
Positive predictive value	87.9 (%)	84.2 (%)
Negative predictive value	88.1 (%)	71.6 (%)
Positive likelihood ratio	9	6.6
Negative likelihood ratio	0.17	0.48

Positivity rates of RAPIRUN and BinaxNow in pneumococcal culture-positive cases by bacterial load, Miller & Jones classification, and Geckler classification

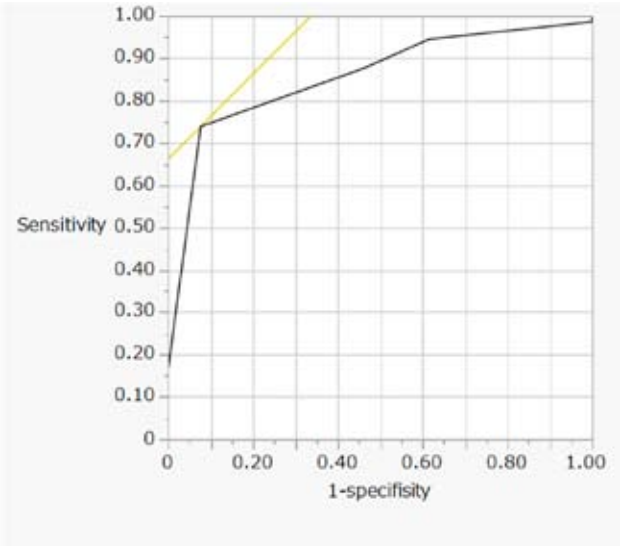
The positivity rates of RAPIRUN and BinaxNow in pneumococcal culture-positive cases are shown in Table 3. The positivity rate of RAPIRUN increased with an increase in the number of bacteria (exact Cochran−Armitage trend test, $p < 0.0001$), but not BinaxNow ($p = 0.275$). RAPIRUN was able to detect *S. pneumoniae* at 10^3 CFU/mL in a single case, and the positivity rate for RAPIRUN exceeded that for BinaxNow at 10^5 CFU/mL or greater. In all 55 cases with detection of 10^7 and 10^8 CFU/mL of *S. pneumoniae*, RAPIRUN was positive in all cases except one. On the other hand, BinaxNow showed a positivity rate of only 50−65.1% among 34 cases. The ROC curves for RAPIRUN and BinaxNow are shown in Figure 1. The AUC was larger for RAPIRUN (0.857) than for BinaxNow (0.559) which indicated superiority of RAPIRUN.

Table 4 shows the positivity rates of both tests according to sputum quality using the Miller & Jones classification. If

Table 3. Positivity rates of RAPIRUN and BinaxNow in pneumococcal culture-positive cases by bacterial load

amount of *S. pneumoniae* (CFU/ml)	culture number of positive cases	RAPIRUN positive number of cases (%)	BinaxNow positive number of cases (%)
10^3	1	1 (100.0)	0 (0.0)
10^4	8	3 (37.5)	4 (50.0)
10^5	7	5 (71.4)	3 (42.9)
10^6	15	10 (66.7)	7 (46.7)
10^7	43	42 (97.7)	28 (65.1)
10^8	12	12 (100.0)	6 (50.0)

a) RAPIRUN



b) BinaxNow

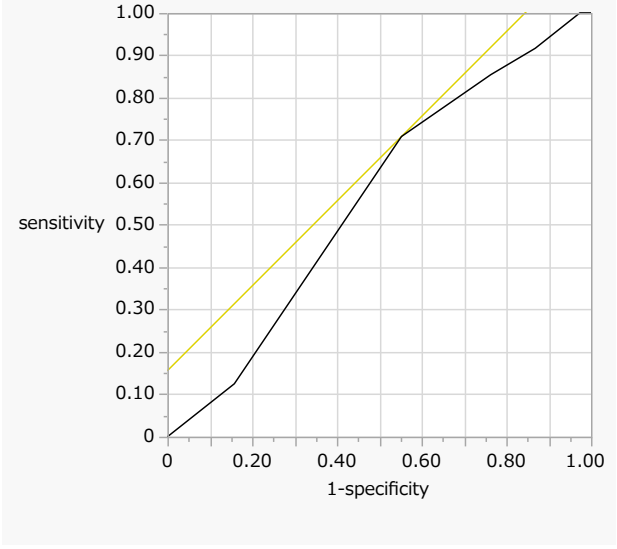


Figure 1. Receiver operating characteristic (ROC) curve for the RAPIRUN (a) and BinaxNow (b). The area under the curve was larger for RAPIUN (0.857) than for BinaxNow (0.559). Black and yellow line present ROC curve and 45-degree tangent line, respectively.

Table 4. Positivity rates of RAPIRUN and BinaxNow in pneumococcal culture-positive cases by the Miller & Jones classification

Miller & Jones classification	culture number of positive cases	RAPIRUN positive number of cases (%)	BinaxNow positive number of cases (%)
M2	17	13 (76.5)	12 (70.6)
P1	22	19 (86.4)	12 (54.5)
P2	26	21 (80.8)	15 (57.7)
P3	21	20 (95.2)	9 (42.9)

the sputum was M2 or more, ≥ 76% of RAPIRUN samples showed a high positivity rate. The positivity rate tended to increase as the purulency of the sputum increased, and P3 sputum showed the highest positivity rate at 95.2%, although there was no statistically significant difference in trend (exact Cochran−Armitage trend test, p = 0.207). On the other hand, BinaxNow showed a positivity rate of 70.6% in M2 sputum, and in contrast to RAPIRUN, the positivity rate decreased as the purulency of the sputum increased; in P3 sputum, the positivity rate decreased to 42.9% (exact Cochran−Armitage trend test, p = 0.154).

Table 5 shows the positivity rates of RAPIRUN and BinaxNow in pneumococcal culture-positive cases, according to the Geckler classification. Generally, a Geckler classification score of 4 or higher is considered appropriate for specimens, and RAPIRUN showed a higher positivity rate than BinaxNow

Table 5. Positivity rates of RAPIRUN and BinaxNow in pneumococcal culture-positive cases by the Geckler classification

Geckler classification	culture number of positive cases	RAPIRUN positive number of cases (%)	BinaxNow positive number of cases (%)
1	2	2 (100.0)	2 (100.0)
2	1	1 (100.0)	1 (100.0)
3	24	21 (87.5)	14 (58.3)
4	26	21 (80.8)	15 (57.7)
5	32	27 (84.4)	15 (46.9)
6	1	1 (100.0)	1 (100.0)

at all classification levels, with no significant difference in trend (data not shown).

False-negative cases of RAPIRUN and BinaxNow in pneumococcal culture-positive cases

The number of cases in which *S. pneumoniae* was not detected by RAPIRUN or BinaxNow despite positive cultures was 13 and 38 for RAPIRUN and BinaxNow, respectively. Eight cases were negative for both RAPIRUN and BinaxNow. A total of 12 (92.3%) of the 13 cases in the RAPIRUN negative arm had bacterial counts below 10^6 CFU/ml, and seven (53.8%) of these cases had counts below 10^5 CFU/mL (Table 6). The RAPIRUN negative rate tended to decrease as the bacterial count of *S. pneumoniae* increased. M2 sputum classified by Miller & Jones, considered unsuitable for specimens, was found in four cases (30.8%). Three cases (23.1%) were evaluated as poor-quality sputum of 3 or less according to the Geckler classification. Gram-positive cocci (GPC) were present in Gram staining in all 13 cases, but GPC tended to be less identified, with 4 cases being 1+ and 7 cases being 2+, and phagocytosis was present in 3 cases.

In contrast, in 38 BinaxNow false-negative cases, the bacterial count did not show the same trend as that of the RAPIRUN arm. M2 sputum was found in five cases (13.2%), while 10 cases (26.3%) were 3 or less by the Geckler classification. Gram-staining findings showed that cases in which a higher number of bacteria was detected in culture tended to have more gram-positive cocci and more phagocytic images (data not shown).

False-positive cases of RAPIRUN and BinaxNOW in pneumococcal culture-negative cases

Six cases were found in which both RAPIRUN and BinaxNow were positive, despite the absence of *S. pneumoniae* in the culture. All but one of the six cases were CAP cases with moderate or higher severity, and four of them had underlying respiratory diseases, such as interstitial pneumonia, COPD, and bronchial asthma. Culture examination revealed *S. agalactiae* in one case. *S. aureus* and *S. epidermidis* were isolated in the other two and one case, respectively. One case of M2, two cases of P1, two cases of P2, and one case of P3 were identified, whereas Gram staining showed GPC 3+ in two cases of P2.

The four RAPIRUN-positive and BinaxNow-negative cases were all CAP cases, with two cases each of severe and moderate disease, respectively. *H. parainfluenzae* and *S. aureus* were detected in two and two cases, respectively. All three cases that tested positive for BinaxNow with negative RAPIRUN results were CAP cases; however, one case had no organisms detected, and *H. parainfluenzae* was detected in two cases. No recent history of respiratory tract infection was noted in the nine cases that tested positive for BinaxNow.

Discussion

This is the first prospective study to compare RAPIRUN and BinaxNow, rapid diagnostic methods for *S. pneumoniae*, in 192 adult patients with respiratory tract infections, and to correlate the results with quantitative culture results. Of the 192 cases, *S. pneumoniae* was detected in culture in 86 cases (44.8%), making this study an appropriate population to compare RAPIRUN and BinaxNow to evaluate how they correlate with quantitative culture tests.

The sensitivity of RAPIRUN and BinaxNow based on culture-positive cases proved that RAPIRUN was superior, with a significant difference in sensitivity. This was also supported with low positive percent agreements value of both tests. The performance of these tests in the current study was similar to that of four previous studies in Japan[12-15].

In a comparison of positive and negative cases of quantitative culture, RAPIRUN, and BinaxNow, it was interesting that the positivity rate of these assays was significantly lower in patients with underlying diseases, especially chronic respiratory diseases such as bronchial asthma, COPD, interstitial pneumonia, and chronic respiratory failure. *H. influenzae* and *H. parainfluenzae*

Table 6. Negative rates of RAPIRUN and BinaxNow in pneumococcal culture-positive cases by culture load

amount of *S. pneumoniae* (CFU/ml)	culture number of positive cases	RAPIRUN positive number of cases (%)	BinaxNow positive number of cases (%)
10^3	1	0 (0.0)	1 (100.0)
10^4	8	5 (62.5)	4 (50.0)
10^5	7	2 (28.6)	4 (57.1)
10^6	15	5 (33.3)	8 (53.3)
10^7	43	1 (2.3)	15 (34.9)
10^8	12	0 (0.0)	6 (50.0)

other than *S. pneumoniae* were dominantly isolated among cases with chronic respiratory diseases and the pattern was not particular compared to that of all cases with no *S. pneumoniae* isolation. Although the apparent reason for this is unclear, one possible reason is that those relatively elderly with chronic respiratory diseases may have more chances to receive pneumococcal vaccines than those without underlying respiratory diseases. However, no clear explanation could be provided because information on vaccination history was not collected in this study. In cases where the detected bacteria considered as the definite causative organism was more than 10^7 CFU/mL, RAPIRUN showed a high positivity rate of 98.2% (54 out of 55 positive cases in this study).

Additionally, RAPIRUN can detect *S. pneumoniae* at 10^3 or 10^4 CFU/mL levels, although the positivity rate is relatively lower. The positivity rate increased with the number of bacteria. In addition, the higher the purulency of sputum in the Miller & Jones classification, the higher the positivity rate of RAPIRUN. The positivity rate of RAPIRUN was also high for all levels in the Geckler classification. These results are reasonable based on the principle of RAPIRUN detecting pneumococcal cell wall antigens in sputum.

BinaxNow was less sensitive than RAPIRUN. None of the BinaxNow-positive cases showed any trend in the Miller & Jones classification, Geckler classification, or Gram staining, which correlated with bacterial abundance. On the other hand, BinaxNow was negative in 21 pneumococcal culture-positive cases with a bacterial load greater than 10^7 CFU/mL. The sputum quality of these cases was good, and not a single negative case was indicated by the RAPIRUN.

Taken together, RAPIRUN is more useful than BinaxNow, especially in cases where the number of bacteria in sputum seems to be large or in cases where the quality of sputum is assured by the Miller & Jones or Geckler classification. Culture tests and active use of RAPIRUN may be recommended when good-quality sputum is obtained from untreated adult patients with respiratory tract infections.

Regarding false-negative cases of the rapid diagnostic methods, all but one of the cases with false-negative results with RAPIRUN had bacterial counts of 10^6 CFU/mL or less. There was a trend toward more M2 cases according to the Miller & Jones classification and fewer cases with 3+ bacterial counts on Gram staining. These results indicate that RAPIRUN may not perform well when the sputum quality is poor.

On the other hand, BinaxNow was negative in 21 cases of pneumococcal culture positive for a high bacterial load of 10^6 CFU/mL or more, and at the same time, despite the presence of clearly high (3+) GPC in Gram staining and phagocytic findings. These cases with negative BinaxNow results showed a completely different trend from that of RAPIRUN. This indicates that pneumonia cases with high bacterial abundance, for which a definite diagnosis of *S. pneumoniae* can be easily obtained in culture tests, may become negative with BinaxNow; therefore, caution is required when using BinaxNow.

Of the 192 cases, 106 had no *S. pneumoniae* isolated in the culture test, 13 were positive for both or only one of RAPIRUN or BinaxNow, which means that 12.2% of the cases were false-positive. The cross-reactivity of RAPIRUN with *Micromonas micros* and *Streptococcus intermedius* has been reported[12]; however, no such organisms were detected in cases in which RAPIRUN showed false-positive results. One case was positive for *Streptococcus agalactiae*, but this case was also positive for BinaxNow, and Gram staining revealed GPC at the 2+ level.

Of the 10 patients with no *S. pneumoniae* isolates in culture tests who tested positive with the RAPIRUN, many had underlying respiratory diseases and also tested positive with the BinaxNow. Additionally, the severity of CAP tended to be relatively high.

False-positive factors for BinaxNow include urinary *Streptococcus mitis* contamination[20], nasopharyngeal colonization with *S. pneumoniae*, especially in infants[10, 11], and a history of previous pneumococcal infection. No cases in this study had an apparent history of recent pneumococcal infection. In addition, no culture tests for *S. mitis* in urine were performed, and the impact of contamination could not be evaluated. Since more than 50% of the cases of pneumonia were not culture-proven for the causative organism, we could not rule out the possibility that true pneumococcal infection was not diagnosed in these false positive cases[6]. From the current study, it should be noted that approximately 10% of respiratory tract infections may result in a false-positive rapid diagnosis of *S. pneumoniae*, which should be recognized along with the limitations of culture testing and rapid diagnostic methods.

One limitation of this study is that, for cases in which no *S. pneumoniae* was isolated in culture tests, the presence of *S. pneumoniae* could have been examined by genetic testing, as in our previous study[13]. Applying such genetic tests may have allowed for a more detailed examination, especially in cases with no culture-positive but positive with RAPIRUN or BinaxNow. In addition, the present study did not require blood culture testing for IPD, which has a higher mortality rate; therefore, we were unable to evaluate the correlation or association with blood culture tests of *S. pneumoniae*.

Conclusion

We compared the performance of the RAPIRUN and BinaxNow rapid immunochromatographic diagnostic methods in adult pneumococcal respiratory infections. As in previous reports, RAPIRUN had a statistically higher diagnostic sensitivity than BinaxNow in cases in which sputum could be collected. In an examination of comparisons with quantitative culture of *S. pneumoniae*, RAPIRUN increased the probability of a positive result with better quality sputum and higher bacterial counts. In contrast, BinaxNow yielded false-negative results in some cases, even when a high load of *S. pneumoniae* was present in the sputum. In adult patients with respiratory tract infections in whom sputum collection is feasible, RAPIRUN should be actively performed to differentiate *S. pneumoniae* infections.

Conflict of interests

Hiroshi Mukae has received research grants fees from Otsuka Pharmaceutical, Co., Ltd. Other authors declared no conflict of interest in this study.

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