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Isolation of Pathogens from Peripheral Pulmonary Blood in Rabbit Pneumonia

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ABSTRACT. The low isolation rate of the etiologic pathogens of human pneumonia has been attributed in part to the use of sputum as the culture specimen. We, therefore, considered that pulmonary arterial blood (PA) might be a more telling specimen for bacteriological isolation and experimented with rabbit pneumonia in order to study its accuracy. Bacterial pneumonia was induced by inoculation of 10^9 cfu/ml of S. aureus (strains 2548 and 5091) through intratracheal tube inserted into the subsegmental bronchi. With this method, the pneumonia was most severe macroscopically, histologically, and bacteriologically at 72 hours after inoculation. At that time, blood samples from the posterior auricular vein (PB) were drawn and, immediately after a thoracotomy, pulmonary arterial blood (PAB) was drawn from the peripheral pulmonary artery. Bronchoalveolar lavage fluid (BALF) was collected with an inserted intratracheal tube and lung homogenates (LH) were sampled from the most severe pneumonic lesions. All these samples were cultured and S. aureus was isolated from 23 out of 35 samples (65%) of LH, 12 out of 37 (32%) of BALF, 5 out of 37 (14%) of PAB and 3 out of 37 (8%) of PB. Contrary to our expectations, the isolation rate of pathogens from PA was low and no diagnostic value in the culturing PA as a culture material in experimental pneumonia.

Key words : experimental pneumonia — pulmonary arterial blood — bronchoalveolar lavage — lung homogenate — isolation rate

Determination of the pathogens in human bacterial pneumonia is principle to the use of an appropriate antimicrobial agent. Isolation, however, is often difficult because sputum, which is the most common specimen, is often unexpectorated and contaminated by oropharyngeal flora and other specimens, such as blood or pleural fluid, are too infrequently sampled to be reliable for positive culture. Therefore, we experimented with rabbit pneumonia, using peripheral pulmonary arterial blood for the bacterial isolation specimen to study its usefulness in making isolation rates more positive.

MATERIALS AND METHODS

A total of 67 male white rabbits (30 in the base study and 37 in the follow-up) weighing about 2 kg, housed in standard cages, and fed with water and food, were used in these studies.

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Two strains (2548,5091) of Staphylococcus aureus (kindly provided by the Fujisawa Pharmaceutical Company Laboratory) were used in the experiments. The bacteria were stored in skim milk at −20℃ until use.

Experimental pneumonia

First, S. aureus strain 2548 was cultured twice in blood agar medium at 37℃ for 24 hours and prepared to 10^9 cfu/ml.

A tracheostomy was performed and 1 ml (10^9 cfu) of S. aureus was infused through a vinyl tube into the rabbit lung to induce staphylococcal pneumonia. Six rabbits were sacrificed at 24, 48, 72, 96, and 120 hours after inoculation, and the lungs were removed. Each time, the lungs of two rabbits were examined macroscopically and histologically. The lungs of the remaining four rabbits were homogenized, cultured in brain-heart-Infusion medium, and quantitatively enumerated.

As the pneumonia was most severe, macroscopically and pathologically, at 72 hours after inoculation, in our next study, we drew 2 ml of posterior auricular vein blood (PB) at that time and, immediately after a thoracotomy, we sampled pulmonary arterial blood (PA), bronchoalveolar lavage (BAL), and lung homogenate (LH) from the most severe lesions. S. aureus strains 2548 and 5091 were grown, prepared and infused into the lungs in the same manner as in the former experiment. All samples were enumerated by quantitative culture in the brain-heart-Infusion medium.

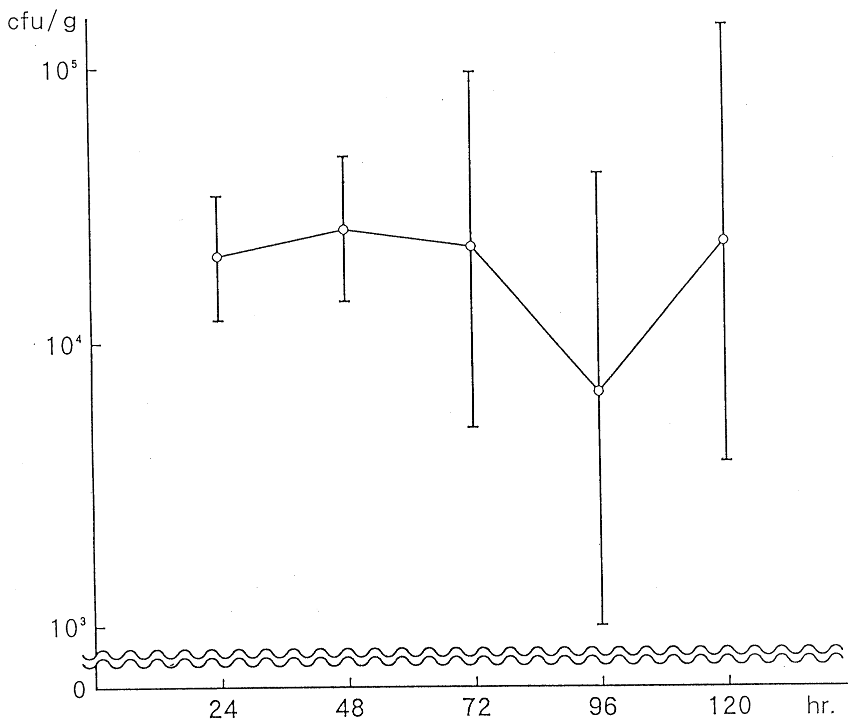

Fig. 1. The number of viable organisms in RLL at varying time after inoculation

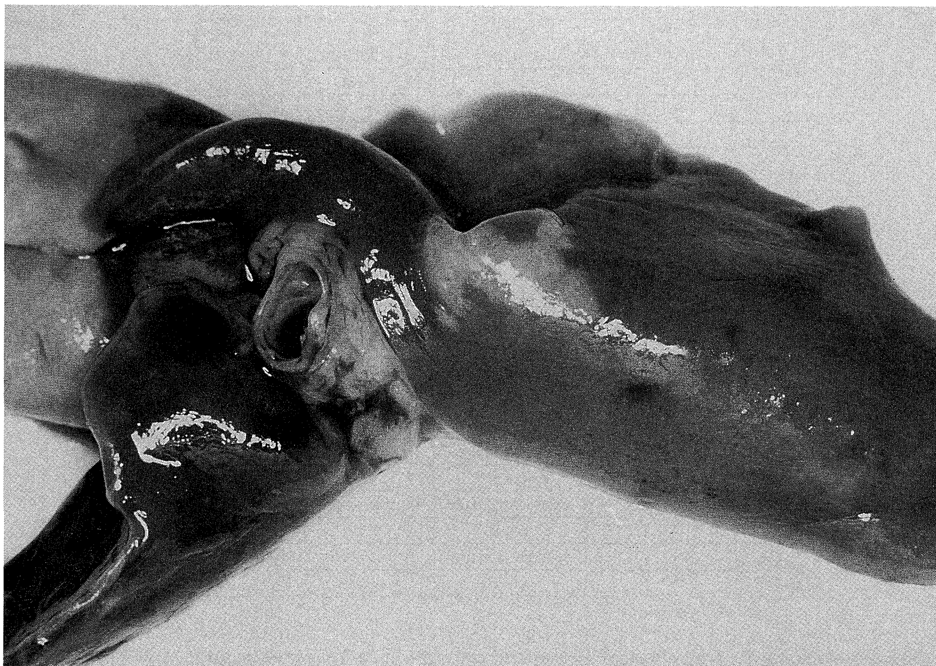

Fig. 2. Macroscopic finding of the right lung at 72 hours after inoculation

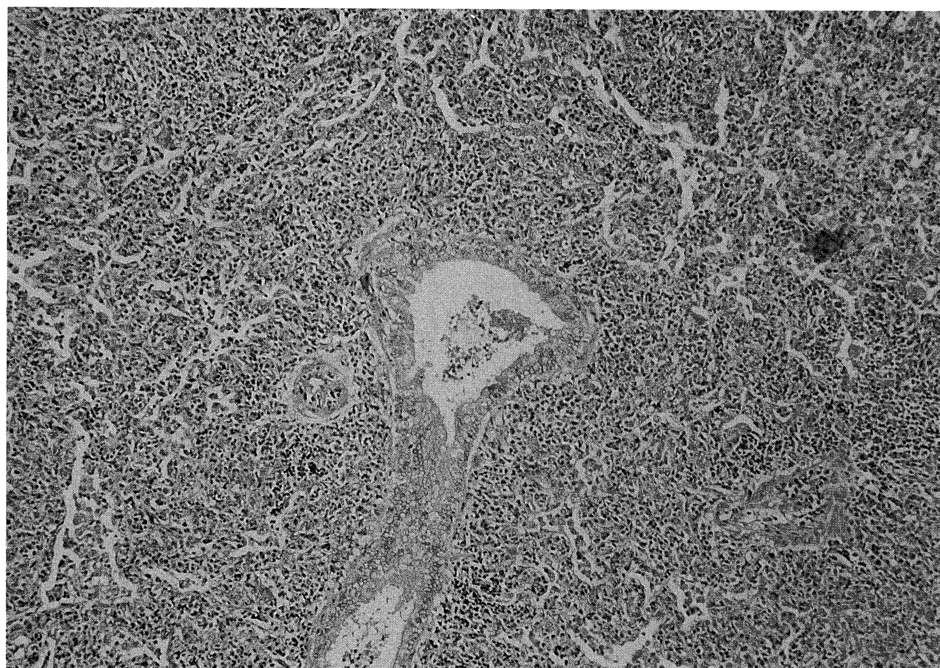

Fig. 3. Histological finding (×100) in the RLL at 72 hours after inoculation

TABLE 1. Isolation of etiological organisms in rabbits with experimental pneumonia (S. aureus 2548)

(n=20)

	PB (cfu/ml)	BAL (cfu/ml)	PA (cfu/ml)	LH (cfu/g)
1	(−)	(−)	(−)	(−)
2	(−)	(−)	(−)	(−)
3	(−)	(−)	(−)	N.D.
4	(−)	(−)	(−)	N.D.
5	(−)	2.1×10^4	(−)	1.1×10^4
6	(−)	(−)	(−)	(−)
7	(−)	(−)	(−)	6.6×10^3
8	(−)	(−)	(−)	(−)
9	(−)	(−)	(−)	3.4×10^4
10	(−)	(−)	(−)	2.7×10^3
11	(−)	(−)	(−)	8.1×10^3
12	2.0×10^2	(−)	(−)	2.2×10^4
13	(−)	2.9×10^4	(−)	1.8×10^3
14	(−)	4.8×10^4	4.7×10^2	1.9×10^5
15	contamination	contamination	(−)	2.1×10^4
16	(−)	(−)	(−)	7.5×10^5
17	(−)	contamination	contamination	2.7×10^4
18	(−)	7.1×10	(−)	4.5×10^4
19	(−)	contamination	(−)	1.7×10^3
20	(−)	(−)	(−)	(−)

N.D.: not done

TABLE 2. Isolation of etiological organisms in rabbits with experimental pneumonia (S. aureus 5091)

(n=17)

	PB (cfu/ml)	BAL (cfu/ml)	PA (cfu/ml)	LH (cfu/g)
1	(−)	(−)	(−)	1.3×10^5
2	(−)	(−)	(−)	(−)
3	(−)	1.3×10	(−)	5.3×10^7
4	(−)	(−)	(−)	(−)
5	(−)	3.0×10	(−)	2.4×10^7
6	(−)	2.5×10^3	5.0×10^3	7.1×10^6
7	(−)	4.6×10^4	5.0×10^2	2.5×10^5
8	(−)	(−)	(−)	1.4×10^2
9	(−)	(−)	(−)	(−)
10	(−)	(−)	(−)	(−)
11	(−)	3.9×10	(−)	1.6×10^4
12	(−)	(−)	(−)	(−)
13	(−)	(−)	(−)	(−)
14	(−)	(−)	(−)	(−)
15	(−)	1.0×10^4	(−)	9.2×10^5
16	1.0×10^2	2.8×10^5	1.3×10^3	1.6×10^5
17	1.0×10	8.7×10^3	6.6×10	3.9×10^6

TABLE 3. Positive ratio of etiological organisms in rabbits with experimental pneumonia

	Total animals	Positive animals	Positive ratio(%)	Positive ratio in rabbits with positive LH (%)
Posterior auricular vein	37	3	8	13
Bronchoalveolar lavage	37	12	32	52
Pulmonary artery	37	5	14	22
Lung homogenates	35	23	65	100

RESULTS

The number of viable organisms from lungs at varying times are shown in Fig. 1. Although the number at 96 hours after inoculation was slightly lower, there were no significant differences among the time periods sampled.

Macroscopic findings of the right lung at 72 hours after bacterial infusion revealed edema, swelling and congestion as shown in Fig. 2. The pneumonia was most severe at this time.

Histological changes in the right lower lobe at 72 hours after inoculation consisted of disruption of the alveoli and cell infiltrations into alveolar and interstitial spaces, as shown in Fig. 3. The number (cfu) of S. aureus strains 2548 and 5091 in the samples are presented in Tables 1 and 2. Staphylococci were isolated from 23 out of 35 samples (65%) of LH, 12 out of 37 (32%) of BALF, 5 out of 37 (14%) of PAB and 3 out of 37 (8%) of PB.

DISCUSSION

The pathogens in human bacterial pneumonia are not often isolated. Blood cultures can be helpful but are not very often positive.[1,2] Pleural fluid cultures may also often be negative. Sputum, which is used most frequently as the culture specimen in respiratory infections, is not always obtainable and may be contaminated by oropharyngeal flora.[3,4] Therefore, some invasive techniques have been used to diagnose pulmonary infections, especially in immunocompromised patients. Cultures of specimens obtained by transtracheal aspirations (TTA) are known to be more helpful and reliable than those of expectorated sputum, but the procedures cause the patient discomfort, and the fluid collected by TTA can be contaminated.[5-7]

Although bronchoalveolar lavage (BAL) and the bronchoscopic protected catheter brush are considered to be valuable techniques, they cause patients more discomfort than TTA, and are difficult to employ with severely ill patients.[8-13] Transthoracic needle aspiration (TNA) is also considered to be a useful method for isolating the pathogens from pneumonic lesions, but it may occasionally be accompanied by dangerous complications such as pneumothorax.[7,14] Therefore, we wished to evaluate the culture of peripheral pulmonary arterial blood as a method of isolating the pathogens from pneumonic lesions, as described in a paper referring to pulmonary microvascular cytology.[15] We thought if the PA isolation rate was similar to those of BAL or TNA, the method could be a useful technique, since obtaining peripheral pulmonary blood through catheterization seems to be both less prone to contamination and less uncomfortable. However, contrary to our expectations, the isolation rate of pathogens from PA in experimental pneumonia was not high, being lower than those of BAL or LH.

In other words there appeared to be no diagnostic value in the culturing of peripheral pulmonary arterial (pulmonary microvascular) blood in pneumonia.

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