

CLINICAL AND BACTERIOLOGICAL STUDY OF BRONCHOPULMONARY INFECTIONS CAUSED BY *STREPTOCOCCUS PNEUMONIAE*

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Between January 1984 and May 1987, 221 episodes of respiratory infection caused by *Streptococcus pneumoniae* (*S. pneumoniae*) were diagnosed in 154 patients by quantitative sputum culture. In 118 (53%) of 221 episodes, *S. pneumoniae* was isolated in pure culture. In 92 (89%) of the remaining 103 episodes, *S. pneumoniae* was associated with *Haemophilus influenzae* (*H. influenzae*) and (or) *Branhamella catarrhalis* (*B. catarrhalis*). A bacterial colony count for *S. pneumoniae* of more than 10^7 CFU/ml was found in 105 (89%) of 118 episodes where *S. pneumoniae* was isolated alone. Ten patients had pneumonia, which was cured in all cases. Fever was present in 29% of the cases in which *S. pneumoniae* was isolated as a single pathogen, while C-reactive protein was positive in 75% and had a 99% correlation coefficient with the white blood cell count in peripheral blood. *S. pneumoniae* strains isolated from 1984-1986 were susceptible to ampicillin, of which the minimal inhibitory concentration (MIC) at 90% was less than 0.1 µg/ml. The MICs of minocycline, erythromycin, ofloxacin and norfloxacin ranged from 0.05-50, 0.013 to more than 100, 1.56-3.13 and 6.25-25 µg/ml, respectively. Fourteen per cent of the strains isolated from 1975-1977 were resistant to minocycline while resistance to erythromycin and clindamycin was not detected. Ten years later, however, the occurrence of pneumococci resistant to minocycline, erythromycin and clindamycin was 23%, 7% and 6%, respectively. In the former period, only 1 of 61 tested strains had intermediate resistance to ampicillin, whereas 6 of 67 strains isolated from 1984-1986 presented intermediate resistance. This study demonstrates the usefulness of quantitative culture of homogenized sputum for determining the causative organism (s) of respiratory infections. Furthermore, according to the *in vivo* and *in vitro* results, it supports the notion that penicillins remain the drug of choice in respiratory infections due to *S. pneumoniae*.

Key words: *Streptococcus pneumoniae*. Quantitative culture of sputum, Bronchopulmonary infections

INTRODUCTION

S. pneumoniae is sometimes seen as an inhabitant of the oropharynx¹⁻³. GRATTEN et al. isolated this bacterium from the upper respiratory tract of all infants examined within the first 3 months of life². Contamination of expectorated sputum by pneumococci from the oropharynx remains an obstacle to establishing accurately the pathogenicity of these organisms³. Using a Kifa-Green catheter,

MATSUMOTO and co-workers⁴) aspirated bronchial secretes from various airway sites and cultured each sample separately. They observed that the causative agents were irregularly distributed in the bronchial tree and that their distribution in sputum might therefore also be irregular. Quantitative culture of homogenized sputum has been observed to lead to isolation of more pathogens than do conventional methods⁵⁻⁷. In addition, it is some-

times very difficult to distinguish colonization of the respiratory tract from true infection. Recent studies have shown, however, that Gram stains of purulent sputum and quantitative culture of homogenized sputum give useful information as to how to solve this embarrassing problem^{6,7}. One of the purposes of this retrospective study is to determine whether the bacteriological diagnosis of respiratory infections due to *S. pneumoniae* corresponds to the existence of clinical disease. The clinical effect of antimicrobial agents which have been used in the management of these infections are also discussed in terms of their MICs.

Before 1967, pneumococci isolated from patients were all believed to be susceptible to penicillin, erythromycin and lincomycin. Since then, however, strains resistant to these drugs are being identified with increasing frequency in many parts of the world⁸⁻¹⁰. Multi-resistance has been described, and meningitis caused by pneumococci with diminished susceptibility to penicillin has proved fatal unless an alternative treatment was implemented^{12,13}. To learn the evolution of the prevalence of ampicillin-, erythromycin-, minocycline- and clindamycin- resistant pneumococci, we compared the susceptibility to these drugs of strains isolated from 1975-1977 with that of strains isolated from 1984-1986.

MATERIALS AND METHODS

Clinical and bacteriological study. Hospital and laboratory records of patients with evidence of respiratory infection caused by *S. pneumoniae* from January 1984 to May 1987 were studied. Selected hospitals included the Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University Hospital and 3 municipal hospitals of Nagasaki Prefecture. Criteria for determining the causative agents, standardized in all 4 hospitals, have already been described¹⁴. Firstly, Gram stains of the purulent sputum showed numerous polymorphonuclear neutrophils, inside which ovoid or lanceolate Gram-positive cocci, arranged in pairs or short chains, and the capsule surrounding each chain were found. Sputa with predominance of epithelial cells are not of bronchial origin and were therefore discarded. Secondly, for quantitative culture of homogenized sputum, *S. pneumoniae* was grown in air on blood agar at 37°C at more than 10⁷ CFU/ml. Optochin sensitivity of the

suspected colonies identified *S. pneumoniae*. Thirdly, disappearance or decrease in number of this bacterium coincided with clinical and bacteriological improvement.

Susceptibility to antibiotics (MIC). Susceptibility to antibiotics of the strains isolated was determined by serial two-fold dilution with an inoculum size of 10⁷ CFU/ml of *S. pneumoniae*. The test medium was Mueller-Hinton agar (BBL) containing 5% rabbit blood. The MIC was defined as the lowest concentration of antibiotic preventing visible growth after overnight cultivation at 37°C. Resistant strains were assessed according to the criteria described previously⁹. Susceptibility to ampicillin, minocycline and erythromycin of *S. pneumoniae* strains isolated from 1984-1986 was studied. Strains isolated from 1985 onwards were tested for susceptibility to ofloxacin and norfloxacin, since these drugs were only available from 1985. Furthermore, we compared the susceptibility to ampicillin, minocycline, erythromycin and clindamycin of strains isolated from 1975-1977 with that of strains isolated from 1984-1986. All antibiotics were supplied as powders of known potency by the manufacturers.

Assessment of clinical response. The overall clinical response to antimicrobial agents was assessed using the following criteria.

Good: clinical and laboratory improvement, no recurrence, no pathogen in sputum or disappearance of sputum in case of acute bronchitis.

Moderate: clear clinical improvement but sputum still containing pathogen.

Poor: no improvement in clinical symptoms or laboratory findings.

RESULTS

Clinical and bacteriological study. One hundred and fifty-four patients were selected for this study (Table 1). The number of men was almost equal to that of women. Mean age was 60 years (range: 19-105). One hundred and thirteen (73%) of 154 patients had acute bronchitis or exacerbation of chronic bronchitis while only 10 patients had pneumonia.

Two hundred and twenty-one episodes of respiratory infections caused by *S. pneumoniae* were diagnosed (Table 2). Of these, 118 (58%) were caused by *S. pneumoniae* isolated as a single pathogen, whereas in the remaining 103 episodes, this

Table 1. Characteristics of patients from 4 hospitals with various respiratory infections associated with *S. pneumoniae*

Hospital code	Number of patients sex M/F	Age average Range	Acute bronchitis	Chronic bronchitis	Bronchi- ectasis	Bronchial asthma + infection	Chronic pulmonary emphysema +infection	Pneumonia
A	36 23/12	60.5 23-82	—	16	10	2	6	2
B	95 51/44	56 19-105	44	39	4	—	5	3
C	13 6/7	71.5 33-84	4	4	—	—	4	1
D	10 3/7	52 45-88	1	5	—	—	—	4
Total	154 83/69	60 19-105	49	64	14	2	15	10

Table 2. Bacteriology of 221 episodes of respiratory infections associated with *S. pneumoniae*

Pathogen	Acute bronchitis	Chronic bronchitis	Bronchi- ectasis	Chronic pulmonary emphysema	Bronchial asthma	Pneumonia	Total
<i>S. pneumoniae</i> alone	28	46	22	14	1	7	118
<i>S. pn</i> & <i>H. influenzae</i>	14	33	9	2			58
<i>S. pn</i> & <i>B. catarrhalis</i>	1	8	3	1	1	1	15
<i>S. pn</i> & <i>P. aeruginosa</i>		1	8				9
<i>S. pn</i> & <i>S. aureus</i>	1						1
<i>S. pn</i> & <i>H. influenzae</i> & <i>B. catarrhalis</i>	5	7	1	3		1	17
<i>S. pn</i> & <i>B. catarrhalis</i> & <i>P. aeruginosa</i>		1	1				2
<i>S. pn</i> & <i>S. aureus</i> & <i>P. aeruginosa</i> & <i>B. catarrhalis</i>						1	1
Total	49	96	44	20	2	10	221
<i>S. pneumoniae</i> alone (%)	57%	48%	50%	70%	50%	70%	53%

bacterium was associated with other organisms. In 92 (89%) of these 103 episodes, *S. pneumoniae* was associated with *H. influenzae* and (or) *B. catarrhalis*. *P. aeruginosa* was present in 9 (41%) of 22 mixed infections in patients with severe bronchiectasis, but only in 2 (4%) of 50 mixed infections in patients with chronic bronchitis. Seven of 10 pneumonias were caused by *S. pneumoniae* alone. In addition, none of the sputa of patients with acute bronchitis yielded *P. aeruginosa*.

Bacterial colony counts of more than 10^7 CFU/ml

were found in 105 (89%) of 118 episodes in which *S. pneumoniae* was isolated alone. In the remaining 13 episodes, the sputum yielded *S. pneumoniae* at less than 10^7 CFU/ml, but the diagnosis of respiratory infection was supported by other parameters (clinical and laboratory findings).

The underlying disorder in 10 patients with pneumonia was chronic pulmonary disease (5), cerebral vascular accident (2), chronic pulmonary disease, cardiac failure and diabetes mellitus (1), old pulmonary tuberculosis (1) and multiple myeloma (1). Blood cultures of the last patient

Table 3. Laboratory findings of the episodes caused by the 3 main pathogens

	<i>S. pneumoniae</i> as single pathogen	<i>S. pneumoniae</i> + <i>H. influenzae</i>	<i>S. pneumoniae</i> + <i>B. catarrhalis</i>	<i>S. pneumoniae</i> + <i>H. influenzae</i> + <i>B. catarrhalis</i>
Fever	33 (29%)	9 (15%)	6 (43%)	4 (25%)
WBC > 8,000/mm ³	30 (40%)	11 (41%)	4 (45%)	2 (29%)
CRP positive	55 (75%)	14 (58%)	8 (89%)	6 (100%)

Table 4. Susceptibility of the strains isolated from 1984 to 1986 for ABPC, MINO, EM and the strains isolated from 1985 to 1986 for OFLX and NFLX

$\mu\text{g/ml}$	0.006	0.013	0.025	0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
ABPC (67)		18	27	16	1	3	1	1								
MINO (113)				2	12	16	7	7	3	8	10	20	19	7		
EM (131)		1	14	55	20	3	3	9	9	4	4		1			8
OFLX (49)									25	24						
NFLX (49)											20	14	15			

were positive for *S. pneumoniae*. Nevertheless, adequate sputum was not obtained.

Clinically, all patients had an increase in frequency and severity of cough (or onset of cough in acute bronchitis) and production of purulent sputum. Fever of more than 37°C was present in 29% of episodes caused by *S. pneumoniae* alone. White blood cell counts of more than 8,000/mm³ in peripheral blood were found in 40% whereas C-reactive protein (CRP) was positive in 75% (Table 3).

CRP and white blood cell counts had a correlation coefficient of 99% (Fig. 1). No case with fever was CRP-negative.

Susceptibility to antibiotics and assessment of clinical results. Penicillins, new quinolones, erythromycin and minocycline were used in 78% of the cases and susceptibility to these drugs of strains isolated from 1984-1986 are shown in Table 4. Ampicillin was the most active. Sixty-one (90%) of 67 tested strains were inhibited by less than 0.1 $\mu\text{g/ml}$ of ampicillin and no growth was found at 0.78 $\mu\text{g/ml}$. Susceptibility to antimicrobial agents other than ampicillin was less favorable. The MICs of minocycline and erythromycin ranged from 0.05-50 $\mu\text{g/ml}$ and from 0.013 to more than 100 $\mu\text{g/ml}$, respectively. Forty-six out of 113 tested strains had MICs of 12.5 $\mu\text{g/ml}$ or more for minocycline while 8 strains had MICs of more than 100 $\mu\text{g/ml}$ for erythromycin. The MICs of ofloxacin and norfloxacin ranged from 1.56-3.13

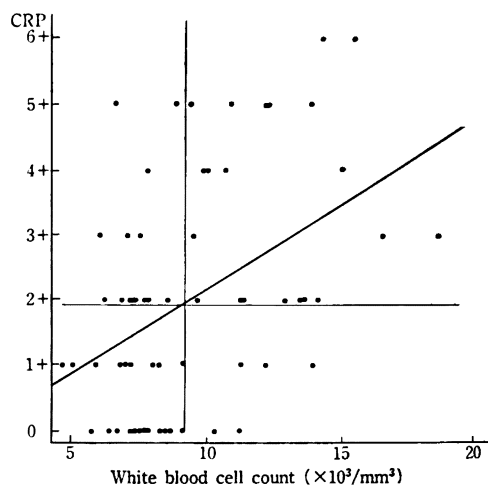


Fig. 1. Correlation between white blood cell count and C-reactive protein

and from 6.25-25 $\mu\text{g/ml}$.

In 53 (45%) of 118 episodes caused by *S. pneumoniae* alone results of sputum culture after treatment were available. They were therefore selected for the assessment of clinical response to the antimicrobial agents used. Amoxicillin and bacampicillin were the penicillins of choice, except in one case where ticarcillin + clavulanic acid was prescribed. Clinical effect was good in 33 (94%) of 35 cases, and moderate in 2 (6%). In 5 of 9 cases treated with new quinolones, *S. pneumoniae* could not be eradicated from sputum. Five cases were treated

Table 5. Overall clinical response to penicillins (amoxicillin or bacampicillin), new quinolones, minocycline and erythromycin

	Good	Moderate	Poor	Total
Penicillins	33 (94%)	2 (6%)		35
New quinolones	4 (45%)	2 (22%)	3 (33%)	9
Minocycline	4 (75%)		1 (25%)	5
Erythromycin	1 (25%)		3 (75%)	4

with minocycline and 4 with erythromycin, but clinical response in general was less favorable (Table 5).

Comparison of antibiotic resistance between *S. pneumoniae* strains isolated from 1975-1977 and those isolated from 1984-1986. Occurrence of *S. pneumoniae* with intermediate resistance to ampicillin and strains resistant to erythromycin, minocycline and clindamycin over the periods 1975-1977 and 1984-1986 are represented in Table 6. In the former period, only 1 of 61 tested strains (2%) had intermediate resistance to ampicillin, whereas 10 years later, 6 (9%) of 67 tested strains had intermediate resistance to ampicillin. Pneumococcal resistance to ampicillin was not detected and the MIC range of the drug remained unchanged in 10 years. Nevertheless, the situation is different regarding the other drugs. In the former period, 9 (14%) of 63 tested strains were resistant to minocycline while no resistance to erythromycin and clindamycin was found. Ten years later, however, occurrence of *S. pneumoniae* resistance to minocycline, erythromycin and clindamycin was 23%, 7% and 6%, respectively. Eight of the strains isolated from 1984-1986 had MICs for erythromycin of more than 100 µg/ml. In addition, 3 of 4 strains resistant to clindamycin had MICs of more than 100 µg/ml.

DISCUSSION

In this study we found that the growth of *S. pneumoniae* at more than 10⁷ CFU/ml correlated in most cases with true respiratory infection. Only 13 (11%) of 118 cases in which *S. pneumoniae* was isolated alone had colony counts of less than 10⁷ CFU/ml. This finding supports our cut-off line of 10⁷ CFU/ml as the threshold of probable infection. Also, quantitative sputum culture provides useful information concerning the efficacy of antimicrobial agents. Prompt decrease in the number of pneumococci correlated with appropriate chemotherapy, whereas the concentration of commensal bacteria could not be correlated with antimicrobial therapy.

LEACH¹⁵⁾, MCFARLANE¹⁶⁾ and HOLMBERG¹⁷⁾ detected pneumococcal Ag in more than 85% of sputum from patients with pneumonia caused by *S. pneumoniae*, whereas recovery of this bacterium from the same specimens did not exceed 50%. While the recovery of *S. pneumoniae* from sputum depends on the suitability of the specimen, the detection of pneumococcal Ag may not depend on the quality of the sputum since recently, KROOK¹⁸⁾ and co-workers detected pneumococcal Ag in 55% of saliva of patients with pneumococcal pneumonia. By doing quantitative cultures of fresh and purulent sputum, however, we succeeded in isolating *S. pneumoniae* from the specimens of patients in whom *S. pneumoniae* infection was suspected from Gram stains.

Very few infections were managed with erythromycin and minocycline, in only a few cases this bacterium was successfully cleared from sputum due to the diminished overall sensitivity of pneumococci to these drugs.

New quinolones have good penetration into bronchial mucosa. Previous studies have shown, however, that these drugs fail to exceed the MIC

Table 6. Comparison of antimicrobial resistance between *S. pneumoniae* strains isolated from 1975 to 1977 to that of the strains isolated from 1984 to 1986

Isolates of 1975-1977				Isolates of 1984-1986			
Number of tested strains	Sensitive strains	Strains with intermediate resistance	Resistant strains	Number of tested strains	Sensitive strains	Strains with intermediate resistance	Resistant strains
ABPC 61	60 (98%)	1 (2%)	0	67	61 (91%)	6 (9%)	0
MINO 63	52 (86%)	—	9 (14%)	113	67 (59%)	—	26 (23%)
EM 62	62 (100%)	—	0	131	122 (93%)	—	9 (7%)
CLDM 62	62 (100%)	—	0	67	62 (94%)	—	4 (6%)

at the site of infection^{16, 20}). This may be the reason why *S. pneumoniae* could not be eradicated from sputum in 55% of our cases.

Amoxicillin and bacampicillin are better absorbed than ampicillin and therefore give higher blood concentrations^{21, 22}. Cases treated with amoxicillin or bacampicillin accounted for 94% of good clinical effect. Sputum of 2 patients treated with amoxicillin yielded *S. pneumoniae* when seen on the third and fifth days of treatment respectively, despite clinical improvement. In both cases, purulency changed from yellowish to whitish. In addition, the bacterial colony count decreased from 4×10^6 CFU/ml and 3×10^7 CFU/ml to 2×10^6 CFU/ml and 5×10^5 CFU/ml, respectively. The overall clinical response might therefore be good if these patients were seen a few days later. Unfortunately, they did not return for follow-up.

Strains of *S. pneumoniae* resistant to erythromycin, minocycline and clindamycin have increased to some extent in the past 10 years. The occurrence of pneumococci with intermediate resistance to ampicillin found in this study remains low and shows no tendency to increase significantly. Resistance of pneumococci to penicillin is in part due to the alteration of penicillin-binding proteins 1 and 2²³). Thus *S. pneumoniae* resistant to penicillin has also a decreased susceptibility to other β -lactam agents, including cephalosporins. *S. pneumoniae* strains with intermediate resistance to ampicillin found in this study have no clinical importance since their persistence in sputum after 7 to 10 days' treatment with amoxicillin or bacampicillin was not observed. Penicillins can therefore still be used in the treatment of pneumococcal infection.

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Dedication

I dedicate the present work to my well loved Edith whose presence was precious for the accom-

plishment of my 5 years' study in Japan.

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肺炎球菌性呼吸器感染症の臨床的・細菌学的研究

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1984年1月から1987年5月までに154人の患者において計221回の肺炎球菌の関与する呼吸器感染症を喀痰定量培養法と臨床像の対比にて診断した。221エピソード中、肺炎球菌のみの単独菌感染症は118エピソード(53%)で、残りの103エピソードのうち92エピソード(90%)はインフルエンザ菌やブランハメラ・カタラーリスとの複数菌感染症であった。肺炎球菌単独感染症118エピソードのうち、喀痰より 10^7 /ml以上の菌数が分離されたのは105エピソード(89%)であった。肺炎球菌性肺炎は10例と少なかったが全例化学療法により治癒した。臨床所見をみると、単独菌感染症例中29%に発熱がみられ、またCRP陽性率は75%であったが、この成績は末梢血の白血球増多とよく一致した。1984年から1986年までの病原性の明確な肺炎球菌はMIC₉₀が0.05 µg/ml以下であった。その他の薬剤ではMINO (0.05~50 µg/ml), EM (0.013~>100 µg/ml), OFLX (1.56~3.13 µg/ml), NFLX (6.25~25 µg/ml)のMICを示した。1975年から1977年までに分離された肺炎球菌のうち14%がMINO耐性であったがEMやCLDM耐性株は認められなかった。しかし10年後には耐性株の占める割合はMINO 23%, EM 7%, CLDM 6%に増加した。一方ABPC耐性菌をみると、前者の時期では1.6%が中等度耐性菌であり、後者の時期では9.0%が中等度耐性を示した。本研究において喀痰定量培養法の有用性が肺炎球菌感染においても証明され、更に肺炎球菌に対するペニシリン剤の有効性が証明された。

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