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Characteristics of *Streptococcus pseudopneumoniae* Isolated from Purulent Sputum Samples

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***Streptococcus pseudopneumoniae* is a recently described streptococcus that is phenotypically and genetically distinct from *Streptococcus pneumoniae* and other viridans streptococci. Key characteristics of *S. pseudopneumoniae* are the absence of a pneumococcal capsule, insolubility in bile, resistance or indeterminate susceptibility to optochin when incubated in 5% CO_2 but susceptibility to optochin when incubated in ambient air, and a positive reaction with the AccuProbe DNA probe hybridization test. The clinical importance of this bacterium is currently unknown. We report the characteristics and associated clinical data of 35 strains of *S. pseudopneumoniae* isolated from sputum samples from 33 patients. All isolates produced a positive result with the NOW *S. pneumoniae* antigen test (Binax, Inc.). No isolate was resistant to penicillin, but 60% were resistant to erythromycin and 77% were resistant to tetracycline. All patients had lower respiratory tract symptoms, 79% had chronic obstructive pulmonary disease (COPD), and 33% had chest radiographic infiltrates. Compared with matched control patients who had *Streptococcus pneumoniae* isolated from sputum, patients with *S. pseudopneumoniae* infection were more likely to have a history of COPD (odds ratio [OR], 5.0; 95% confidence interval [CI], 1.67 to 20.11) or exacerbation of COPD (OR, 6.5; 95% CI, 2.61 to 16.20). Further research is needed to better characterize the epidemiology of *S. pseudopneumoniae* colonization and the role of *S. pseudopneumoniae* in COPD and other diseases.**

Streptococcus pseudopneumoniae is a recently described streptococcus that is phenotypically and genetically distinct from *Streptococcus pneumoniae* and other viridans streptococci (1, 5). DNA-DNA homology studies suggest that this species is a member of the *Streptococcus mitis-Streptococcus oralis* group (1), and it is likely that this species is similar to other strains previously described by several investigators as atypical pneumococci (4, 9, 12). *S. pseudopneumoniae* can be differentiated from *S. pneumoniae* and *S. mitis* by the absence of a pneumococcal capsule, demonstration of insolubility in bile, resistance or indeterminate susceptibility to optochin when incubated in 5% CO_2 but susceptibility to optochin when incubated in ambient air, and a positive reaction with a commercial DNA probe hybridization test (AccuProbe *Streptococcus pneumoniae* culture identification test; Gen-Probe, San Diego, CA).

Although the first-described isolates of *S. pseudopneumoniae* came from lower respiratory tract samples (1), the pathogenic potential and clinical importance of this bacterium are still undetermined. We report the characteristics and associated clinical data of 35 strains of *S. pseudopneumoniae* isolated from sputum samples.

MATERIALS AND METHODS

Isolates. Since May 2001, we have been collecting consecutive alpha-hemolytic streptococcal strains isolated from sputum samples sent to our diagnostic laboratory. Only strains isolated from good-quality samples (>25 leukocytes and ≤10 squamous epithelial cells/×100 field) showing a Gram stain and culture predominance were archived. Isolates were identified as *S. pseudopneumoniae* on the basis of tests for pneumococcal capsule, bile solubility, optochin susceptibility, and AccuProbe DNA hybridization.

Bile solubility test. 0.5 ml of 2% deoxycholate was added to 0.5-ml suspensions of each isolate prepared in phosphate-buffered saline (PBS) and incubated at 35°C for 2 h. A positive test was indicated by visible clearing of the suspension.

Optochin susceptibility test. Sheep blood agar plates were inoculated with colonies from cultures grown overnight, and a 5-μg optochin disk was placed in the center of each inoculum. Each isolate was then incubated for 18 to 24 h at 35°C in both 5% CO_2 and ambient air environments. Optochin susceptibility was defined as a zone of inhibition of ≥14 mm.

DNA probe hybridization test. The AccuProbe *Streptococcus pneumoniae* culture identification test (Gen-Probe, San Diego, CA) was performed according to the manufacturer's instructions.

Detection of pneumococcal capsule. The Quellung test was used to detect the presence of a pneumococcal capsule. A light suspension of bacteria in PBS was air dried on a slide to which 5 μl polyvalent pneumococcal antisera (Omni Serum, Statens Serum Institut, Copenhagen, Denmark) diluted 1:4 in PBS and a small drop of methylene blue were added. A positive test was indicated by the presence of a sharply demarcated capsule when observed under oil immersion microscopy.

Rapid ID32 Strep identification system. The Rapid ID32 Strep identification system (bioMérieux, France) test was performed according to the manufacturer's instructions.

Pneumolysin gene (*ply*) PCR. The presence of the *ply* gene in extracted DNA from isolates was determined by PCR as previously described (7).

NOW *S. pneumoniae* immunochromatographic antigen test. The NOW *S. pneumoniae* immunochromatographic antigen test (Binax, Portland, ME) detects the C-polysaccharide cell wall antigen common to all *S. pneumoniae* strains. A single colony was touched by a swab, which was then placed into the test device. The test was then performed according to the manufacturer's instructions, with 6 drops of buffer solution added. A positive test result was indicated by the detection of both sample and control lines.

Antimicrobial susceptibility testing. Antimicrobial susceptibilities were determined by disk diffusion according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2). Penicillin MICs were determined by the Etest (AB BIODISK, Solna, Sweden).

Clinical data. Demographic, clinical, and laboratory characteristics of all patients who had *S. pseudopneumoniae* isolated from sputum were obtained from a review of clinical records. For comparison, two control patients, matched for

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TABLE 1. Phenotypic and genotypic test results for strains of *S. pseudopneumoniae*

Isolate[a]	Optochin zone size (mm)[b]		Bile soluble[e]	AccuProbe result[f]	Ply[c]	NOW antigen test result[f]	Rapid ID32 Strep result	
	CO_2	O_2					Profile	Identification
22	14	32	−	+	+	+	64032441100	86.7% *S. oralis* II
28	10	32	−	+	+	+	40012441100	65.5% *S. mitis* I 33.4% *S. oralis* II
35	14	24	−	+	+	+	04012541100	73.8% *S. mitis* I 23.2% *S. oralis* II
78	14	28	−	+	+	+	24002500000	36.7% *Gemella haemolysans*
83	12	34	−	+	+	+	44012241100	95.9% *S. oralis* II
114	10	24	−	+	−	+	44012541100	54.8% *S. oralis* II 42.5% *S. mitis* I
134	6	30	−	+	+	+	44132544100	Unacceptable profile
147	6	26	−	+	−	+	04112541100	98.2% *S. mitis* I
153	12	34	−	+	−	+	44112641100	87.3% *S. oralis* II
169	13	32	−	+	+	+	44112441100	93.4% *S. mitis* I
170	13	22	−	+	+	+	64002400000	46.2% *S. mitis* II
178	8	26	−	+	+	+	44112641100	87.3% *S. oralis* II
226	10	26	−	+	+	+	04012541100	73.8% *S. mitis* I 23.2% *S. oralis* II
288	8	22	−	+	−	+	64036641100	98.3% *S. intermedius*
369	6	26	−	+	+	+	44012541100	54.8% *S. oralis* II 42.5% *S. mitis* I
407	6	26	−	+	+	+	04002500000	48.5% *G. haemolysans*
418	8	22	−	+	+	+	44112441100	93.4% *S. mitis* I
431	12	46	−	+	+	+	44012441100	56.1% *S. oralis* II 43.5% *S. mitis* I
434	8	22	−	+	+	+	24332441100	96.9% *S. sanguis* II
441	13	36	−	+	+	+	64112544100	Unacceptable profile
445	6	36	−	+	+	+	64012441100	91.9% *S. oralis* II
457	10	26	−	+	+	+	64132541100	Unacceptable profile
458	6	24	−	+	+	+	64012560100	Unacceptable profile
474	12	26	−	+	+	+	44012441100	56.1% *S. oralis* II 43.5% *S. mitis* I
479	10	28	−	+	−	+	64112441100	56.3% *S. mitis* I 43.4% *S. oralis* II
480	6	36	−	+	+	+	04002401100	88.6% *G. morbillorum*
490	6	36	−	+	+	+	44012441100	56.1% *S. oralis* II 43.5% *S. mitis* I
499	10	36	−	+	+	+	44012441100	56.1% *S. oralis* II 43.5% *S. mitis* I
554	8	24	−	+	+	+	44112441100	93.4% *S. mitis* I
589	10	26	−	+	+	+	44002400000	Unacceptable profile
669	9	26	−	+	+	+	44112441100	93.4% *S. mitis* I
705	10	30	−	+	+	+	44012401100	77.0% *S. oralis* II
747	6	21	−	+	+	+	44312441100	93.4% *S. mitis* I
773	8	26	−	+	+	+	44012441100	56.1% *S. oralis* II 43.5% *S. mitis* I
785	6	24	−	+	+	+	24012441100	71.4% *S. oralis* II 20.4% *S. mitis* I
Reference strain[d]	13	22	−	+	+	+	44012441100	56.1% *S. oralis* II 43.5% *S. mitis* I

[a] Isolate 28, 83, and 490 are from different sputum samples from the same patient.
[b] Optochin susceptibility in 5% CO_2 and in ambient (O_2) environments.
[c] Ply, pneumolysin gene. +, pneumolysin gene was detected; −, pneumolysin gene was not detected.
[d] *S. pseudopneumoniae* NZRM4311 (ATCC BAA-960).
[e] −, isolate was not bile soluble.
[f] +, positive test result.

age and sex, were obtained for each case. These controls were identified from the same streptococcal database by selecting the next two patients chronologically after the case who were of the same gender and within 5 years of age and who did not have *S. pseudopneumoniae* isolated from sputum. The same data as for the cases were collected from the control patients. Mantel-Haenszel matched odds ratios were calculated for comparisons between cases and controls.

The study was approved by the Canterbury Ethics Committee.

RESULTS

Of 805 consecutive isolates collected between May 2001 and May 2004, 35 isolates (4%) from 33 patients were identified as *S. pseudopneumoniae*. All isolates lacked pneumococcal capsules, were bile insoluble, were resistant or had indeterminate

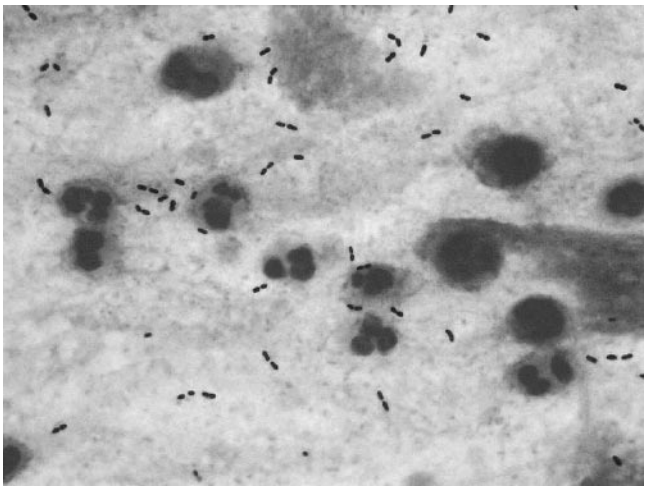

FIG. 1. Typical Gram stain appearance of a sputum smear that grew *S. pseudopneumoniae* as the sole pathogen.

susceptibility to optochin when incubated in CO_2 but were susceptible to optochin when incubated in an ambient environment, and had positive reactions with the AccuProbe DNA probe hybridization test. In addition, the colonial morphology of all isolates was similar to that of a type strain of *S. pseudopneumoniae* (NZRM4311; ATCC BAA-960). *S. pseudopneumoniae* colonies grown on sheep blood agar are typically small (up to 1 mm in diameter after 24 h of incubation), smooth, shiny, and domed, with entire edges. Occasional colonies have depressed centers, causing them to appear as a smaller version of the draftsmen colonies of *S. pneumoniae*. Other phenotypic and genotypic test results are shown in Table 1. All *S. pseudopneumoniae* isolates were collected before the first description of this species and had been originally identified as either alpha-hemolytic streptococci (33 isolates) or *S. pneumoniae* (2 isolates). Figure 1 shows a typical sputum Gram stain smear from a sample that grew *S. pseudopneumoniae* as the sole pathogen and illustrates the similar appearance to *S. pneumoniae*.

Of the 35 *S. pseudopneumoniae* isolates, none was resistant to penicillin or vancomycin, 21 (60%) were resistant to erythromycin, 27 (77%) were resistant to tetracycline, and 4 (11%) were resistant to cotrimoxazole. The penicillin MICs ranged from <0.016 to 1.5 μg/ml (MIC at which 50% of the isolates are inhibited, 0.016 μg/ml).

Clinical, laboratory, and treatment data from the 33 patients with positive sputum cultures for *S. pseudopneumoniae* are presented in Table 2. One patient had *S. pseudopneumoniae* isolated on three separate occasions over 17 months; the three isolates had identical phenotypic and genotypic characteristics,

TABLE 2. Characteristics of patients with *S. pseudopneumoniae* isolated from sputum

Patient	Age (yr)	Sex	Clinical presentation	Current or ex-smoker[c]	Chest X-ray infiltrate[a]	Other bacteria in sputum	Antibiotic treatment[b]	Outcome
1	64	F	Exacerbation of COPD	+	−	*H. influenzae*	Amx/Cla	Improved
2	82	M	Motor neuron disease, COPD	+	+		None	Improved
3	63	M	Cough, ischemic heart disease, diabetes	−	+		Amx/Cla	Readmitted with pneumonia
4	63	M	Amaurosis fugax, cough, COPD	+	−		None	Improved
5	89	F	Lobar pneumonia, COPD	+	+		Amoxicillin	Improved
6	80	M	Ischemic heart disease, cough, COPD	+	+		None	Readmitted with pneumonia
7	51	F	Exacerbation of COPD	+	−		Ciprofloxacin	Improved
8	15	M	Bronchiectasis	−	−	*H. influenzae*	Cefuroxime	Improved
9	78	F	Pubic ramus fractures, cough, COPD	+	−	*H. influenzae*	Amx/Cla	Improved
10	57	M	Exacerbation of COPD	+	+		Amx/Cla	Improved
11	78	M	Fractures, cough, COPD	+	+		Amx/Cla	Improved
12	86	M	Exacerbation of COPD	+	−	*H. influenzae*	Amoxicillin	Improved
13	78	M	Exacerbation of COPD	+	+		Clarithromycin	Improved
14	77	M	Exacerbation of COPD	+	−		Erythromycin	Improved
15	62	M	Exacerbation of COPD	+	ND	*H. influenzae*	Amx/Cla	Improved
16	55	F	Ischemic heart disease, cough	+	−		None	Improved
17	81	F	Exacerbation of COPD	+	−		Amoxicillin	Improved
18	68	M	Exacerbation of COPD	+	−	*H. influenzae*	Amoxicillin	Improved
19	52	F	Bronchiectasis	−	−	*H. influenzae*	Amoxicillin	Improved
20	77	M	Exacerbation of COPD	+	+	*H. influenzae*	Roxythromycin	Improved
21	28	F	Asthma	+	ND		Unknown	Unknown
22	74	M	Lung cancer, COPD	+	−	*H. influenzae* *M. catarrhalis*	None	Improved
23	69	F	Exacerbation of COPD	+	−		Amx/Cla	Improved
24	53	M	Bronchiectasis	+	ND		None	Improved
25	50	F	Exacerbation of COPD	+	+		Amx/Cla	Improved
26	61	F	Exacerbation of COPD	+	−	*H. influenzae* *Pseudomonas aeruginosa*	Amx/Cla	Improved
27	77	M	Renal cell carcinoma, COPD	+	−		Roxythromycin	Improved
28	53	M	Asthma, COPD	+	−		Unknown	Unknown
29	66	F	Exacerbation of COPD	+	−		None	Improved
30	72	M	Exacerbation of COPD	+	−		Ciprofloxacin	Improved
31	74	F	Exacerbation of COPD	+	−		None	Improved
32	75	M	Prostate cancer, cough	+	+		Amx/Cla	Improved
33	63	F	Exacerbation of COPD	+	−		Amx/Cla	Improved

[a] ND, not done; −, chest infiltrates were not detected; +, chest infiltrates were detected.
[b] Amx/Cla, amoxicillin-clavulanic acid.
[c] +, current or ex-smoker; −, not a current or ex-smoker.

TABLE 3. Clinical features of patients who had *S. pseudopneumoniae* cultured from sputum compared with control patients who had *S. pneumoniae* cultured from sputum

Clinical feature	No. of patients with characteristic/no. with available information (%)		Odds ratio (95% CI)[a]	*P* value
	Cases	Controls		
History of COPD	26/33 (79)	36/66 (55)	5.00 (1.67 to 20.11)	0.002
Exacerbation of COPD	17/33 (52)	12/66 (18)	6.50 (2.61 to 16.20)	0.0001
History of bronchiectasis	3/33 (9)	4/66 (6)	1.50 (0.36 to 7.23)	0.75
History of asthma	2/33 (6)	4/66 (6)	1.00 (0.07 to 13.80)	1.00
Current or ex-smoker	30/33 (91)	50/64 (78)	3.00 (0.91 to 12.76)	0.08
Chest radiographic infiltrates	10/30 (33)	29/54 (54)	0.45 (0.18 to 1.03)	0.06

[a] CI, confidence interval.

apart from a slightly different Rapid ID32 profile (Table 1), and only clinical data related to the first isolation were analyzed. The median age of the patients was 68 years (range, 15 to 89), 19 patients (58%) were male, and 30 patients (91%) were inpatients. All patients had lower respiratory tract symptoms with cough, and exacerbation of chronic obstructive pulmonary disease (COPD) was the primary reason for presentation in 17 (52%) cases. Spirometry data were available for 17 of the 26 cases with a history of COPD: 4 were of severity II, 5 were of severity III, and 8 were of severity IV according to the GOLD criteria (6). For those patients who had data available, 9/31 (29%) had peripheral leukocyte counts of $>11 \times 10^9$ cells/liter, and only 3/30 (10%) had a temperature of >37.5°C on presentation. In each of the 10 patients with mixed infections, *S. pseudopneumoniae* was clearly the predominant organism isolated. There were no observed differences between patients with mixed infections and other patients (data not shown). All the control patients had positive sputum cultures for *S. pneumoniae*, and 20 patients (30%) had positive sputum cultures for *S. pneumoniae* mixed with other respiratory pathogens (mainly *Haemophilus influenzae* or *Moraxella catarrhalis*). Table 3 compares some clinical features of cases and controls. A history of COPD and exacerbation of COPD as a presenting feature were both significantly more common among cases than among controls.

DISCUSSION

Our findings indicate that *S. pseudopneumoniae* can be isolated from a small proportion of sputum samples and that these strains had been previously identified as either viridans streptococci contaminants or as unusual pneumococci. Although sharing many features in common with *S. pneumoniae* and *S. mitis*, *S. pseudopneumoniae* can be readily identified in a clinical laboratory by the combination of some simple phenotypic tests, particularly tests for optochin susceptibility and bile solubility. Interestingly, *S. pseudopneumoniae*, like *S. mitis* (8), produces a positive result with the NOW *S. pneumoniae* antigen test, indicating that the antigen is shared between the species. Unlike the findings previously reported by Arbique et al. (1), who detected the pneumolysin gene in all of their *S. pseudopneumoniae* strains, five of our isolates tested negative for this gene. In addition, we documented a high rate of resistance to erythromycin and tetracycline among the *S. pseudopneumoniae* isolates. Recently, a single strain of *S. pseudopneumoniae* was found to be resistant to erythromycin and tetracycline due to the presence of *mef*(E) and *tet*(O) genes, respectively (3).

It can be difficult to determine whether a microorganism is a respiratory pathogen or whether it is simply a respiratory tract colonizer. This is especially so in the setting of COPD, where lower respiratory tract colonization with potential pathogens may occur even during times of clinical stability (10). In the present study, all strains of *S. pseudopneumoniae* were isolated as the predominant or only microorganism from good-quality purulent sputum samples obtained from patients with lower respiratory tract symptoms. Furthermore, in all cases, the sputum smear Gram stain results indicated the presence of *Streptococcus* species as the predominant bacteria. These findings are difficult to ignore and provide supporting evidence of a potential pathogenic role of *S. pseudopneumoniae*. In addition, approximately one-third of these patients had chest radiographic infiltrates, and one-third had peripheral leukocytosis. However, all these findings do not constitute definitive evidence of the pathogenic potential of *S. pseudopneumoniae*, and more data are required to support this notion. It is interesting that about one-quarter of patients with *S. pseudopneumoniae* in sputum were not treated with antibiotics and that all but one of these patients clinically improved.

Our preliminary data indicate that the isolation of *S. pseudopneumoniae* from sputum was associated with both a history of COPD and exacerbation of COPD. Over three-quarters of cases had documented COPD, and for at least half of all cases, exacerbation of COPD was the primary reason for seeking medical attention. Due to the large proportion of cases with a history of COPD, we performed a case control study to determine whether this result was a true association or whether it just reflected the background rate of COPD among patients who submitted sputum samples to our tertiary hospital laboratory. The findings of this study indicate that COPD was significantly more common among patients with *S. pseudopneumoniae* isolated from sputum than among the control group of patients who did not.

This study has several limitations, and we must stress the preliminary nature of our findings. We relied on information retrospectively retrieved from clinical records. Some information from the time of sputum collection was sparse, limiting the ability to obtain a detailed clinical picture for some patients. We may have underestimated the number of patients with COPD and other respiratory diseases owing to missing information. To better characterize the potential role of *S. pseudopneumoniae* in the exacerbation of COPD, it will be essential to examine COPD patients with exacerbations and compare them to those who do not with regard to isolation of this organism. Ideally, it would be important to monitor a cohort of patients with COPD and examine them during both exacerbations and times of clinical stability. The appearance of *S. pseudopneumoniae* or a new strain of *S. pseudopneumoniae* during exacerbations would support the widely accepted hypothesis that acquisition of a new strain of bacteria plays a causative role in exacerbation of COPD (11). Further work also needs to focus on the natural habitat of *S. pseudopneumoniae*, the epidemiology of *S. pseudopneumoniae* colonization, and the role of *S.*

pseudopneumoniae in infections outside the setting of COPD, including pneumonia.

S. pseudopneumoniae isolates have now been isolated from North America and New Zealand, and it is likely that some of the so-called atypical pneumococci reported from Europe (4, 9, 12) are the same species. Increased awareness of this species will help to better determine its prevalence and clinical importance. Preliminary data from this study should prompt further research to characterize the role of *S. pseudopneumoniae* in COPD.

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