Eur Respir J
1988, 1, 415–420

The epidemiology of respiratory tract pathogens in Southern Netherlands

B.I. Davies*, F.P.V. Maesen**

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ABSTRACT: Results of sputum cultures and susceptibility tests have been reviewed for the years 1977–1986, with approximately 1,700 positive cultures per year. *Haemophilus influenzae* always occupied the first place, being grown in 65% of positive specimens in 1977 and 37% in 1986. *Streptococcus pneumoniae* occupied the second place, rising from 17 to 27% in the same period, during which *Branhamella catarrhalis* rose from 5 to 26%. Based on the frequency of occurrence of individual bacterial species in the sputum cultures and on the percentage of strains resistant to ampicillin, cotrimoxazole, doxycycline, erythromycin and ofloxacin, theoretical chances of failure of blind therapy were calculated and found to be most advantageous for ofloxacin and cotrimoxazole. No explanation could be given for the increase in β-lactamase production by *B. catarrhalis*, but the disappearance of *Haemophilus parainfluenzae* may have been due to improvements in the identification procedure.

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Departments of Medical Microbiology* and Respiratory Diseases**, De Wever Ziekenhuis, Heerlen, The Netherlands.

Correspondence: B.I. Davies, Department of Medical Microbiology, De Wever Ziekenhuis, 6401 CX Heerlen, The Netherlands.

Keywords: Antibiotic susceptibilities; epidemiology; respiratory pathogens.

Received: August 24, 1987; accepted after revision December 19, 1987.

Little or no detail is known about quantitative and qualitative changes in the occurrence of bacterial pathogens in the respiratory tract during the course of time. For example, changes in the frequency of occurrence of different organisms and changes in their sensitivities to antimicrobial agents have seldom been documented. In the Regional Public Health Laboratory of the De Wever Ziekenhuis in Heerlen, The Netherlands, which has a surrounding population of approximately 300,000 in the 'Old Mining District', all the identities and sensitivities of the bacteria cultured from sputum between 1977 and 1986 have been carefully registered. Considering the geographical situation of this district and the way it adjoins and is nearly surrounded by Belgium and Germany, (fig. 1), the region lends itself to epidemiological studies as it appears to be almost an island.

Materials and methods

In the period between 1977 and the end of 1986 between 3,000 and 5,000 sputum specimens were submitted each year for bacteriological examination. Approximately three quarters had their origin in the De Wever Hospital and the other quarter were predominantly from the outpatient departments. The great majority of all the sputum samples came from patients with various degrees of chronic obstructive lung disease, in whom recurrent episodes of purulent infection are well known. A relatively small number came from hospital inpatients with respiratory infections such as postoperative pneumonia, or pneumonia acquired during assisted respiration. It is considered normal practice in most Dutch hospitals to take cultures from all patients with purulent sputum. Only a small number of specimens were submitted by the general practitioners in the district.

All sputum samples were first inspected macroscopically and only those which appeared to consist of genuine sputum were examined further. These specimens were examined by the method originally described by MULDER *et al.* [1], which we have modified slightly [2], and were washed in three changes of sterile physiological saline in a safety cabinet. Smears were then made for Gram staining and cultures were carried out according to conventional techniques. The micro-organisms cultured were identified by standard methods [3] although the porphyrin test [4, 5] for the identification of *H. influenzae* and *H. parainfluenzae* was only introduced in 1982 as an addition to the conventional X and V factor dependence tests. The susceptibilities to doxycycline, cotrimoxazole and erythromycin (and, in recent times, ofloxacin) were measured by disc diffusion tests on appropriate culture media free from antagonists to trimethoprim and sulphonamides. In addition, minimum inhibitory concentrations were measured for ampicillin for all respiratory isolates. Whenever *Haemophilus influenzae*, *Haemophilus parainfluenzae* or *Branhamella catarrhalis* were cultured, β-lactamase activity was assessed using nitrocefin solution [6] (up to 1981), and nitrocefin discs [2, 7] since that time.

Results

As shown in table 1, the positive sputum cultures averaged approximately 1,400 to 1,800 per year, or

Fig. 1. Map of Southern Netherlands. Old Mining District is shown hatched, with Germany lying to the East and Belgium to the South and the West.

approximately 40% of the specimens examined. Approximately 70% of the total pathogen flora consisted of (in order of importance) *H. influenzae*, *Streptococcus pneumoniae* and *B. catarrhalis*. *Haemophilus parainfluenzae*, which still amounted to at least 13% of the pathogenic flora in 1980, had practically disappeared from the scene in 1983. *H. influenzae*, which accounted for 65% of the total isolates in 1977, fell to approximately 35% in 1980 and has remained impressively constant since that time. In contrast, the number of *S. pneumoniae* strains has increased in recent years and this organism now accounts for 27% of the total bacterial population. However, these figures are much less impressive than the enormous increase in the frequency of occurrence of *Branhamella catarrhalis*, which was scarcely 5% in 1977 but now accounts for 26% of the respiratory tract pathogens cultured. *Pseudomonas aeruginosa* has also increased very slightly but still does not exceed 10% of the total number of bacteria cultured. The remaining 25% of the pathogens cultured consisted of various other organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, etc.

When the results of the sputum cultures from hospitalized patients were compared with those from patients attending the outpatient clinic (table 2) the differences in the bacterial flora were only small. *Haemophilus influenzae* was slightly less common in inpatients than in outpatients (37.3 against 50%) whereas *Branhamella catarrhalis* occurred more often in the specimens from the hospitalized patients (29.7 compared to 21.8%). It is also interesting to note that *Pseudomonas aeruginosa* was only slightly more common in the clinical patients (9.2%) than in patients attending the outpatient departments (6.2% of positive sputum cultures) and this was also the case with *Staphylococcus aureus* (table 2). A mixed flora of pathogens was found in approximately 15% of all patients (table 3). Once again, there was no great difference between the outpatient and inpatient culture results, although the combination of *H. influenzae*, *S. pneumoniae* and *B. catarrhalis* (the 'big three') was noted in 3.9% of outpatient samples but in only 1.8% of inpatient specimens.

As far as the susceptibility of these bacteria to antimicrobial agents is concerned, we can see from table 4 (which includes data from 1987) that *Haemophilus influenzae* was relatively insensitive to erythromycin, but usually susceptible to doxycycline, ampicillin and cotrimoxazole. Paradoxically, the susceptibility of the bacteria from the outpatient

Table 1. - Results of all sputum cultures 1977-1986

Year studied:	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986
specimens studied	3,240	3,994	3,827	4,630	3,829	3,512	3,914	4,179	5,159	4,881
positive cultures	1,315	1,380	1,422	1,556	1,785	1,591	1,717	1,876	1,736	1,717
percentage positive	40.6	34.6	37.2	33.6	46.6	45.3	43.9	44.9	33.7	35.2
*Bacteria cultured:										
H. influenzae	65	51	40	32	35	36	37	37	34	37
S. pneumoniae	17	20	22	19	17	25	26	25	21	27
B. catarrhalis	5	12	13	16	17	20	22	15	18	26
H. parainfluenzae	7	9	8	13	12	8	1	-	1	-
P. aeruginosa	2	3	6	5	7	13	11	10	11	6
All other organisms	14	15	16	17	24	24	20	20	26	25

*Results expressed as percentages of positive cultures in each year. Because of mixed cultures, these percentages generally exceed 100.

Table 2. - 2,091 bacterial strains from 1,623 sputum cultures in 1986

Organism	Hospital Outpatients n	%	Hospital Inpatients n	%	Total n	%
H. influenzae	193	(50.0)	461	(37.3)	654	(40.3)
S. pneumoniae	104	(26.9)	382	(30.9)	486	(29.9)
B. catarrhalis	84	(21.8)	367	(29.7)	451	(27.8)
P. aeruginosa	24	(6.2)	114	(9.2)	138	(8.5)
Staph. aureus	16	(4.1)	107	(8.6)	123	(7.6)
Various bacteria	68	(17.6)	171	(13.8)	239	(14.7)
Total positive cultures:	386	(100)	1,237	(100)	1,623	(100)
Total isolates:	489		1,602		2,091	

N.B. because of mixed cultures, these percentages add up to more than 100; n: number of sputum cultures from which the organism was isolated.

Table 3. - Sputum culture results in 1986

	Hospital Outpatients n	%	Hospital Inpatients n	%	Total n	%
H. influenzae[*]	133	(34.5)	288	(23.3)	421	(25.9)
S. pneumoniae[*]	53	(13.7)	208	(16.8)	261	(16.0)
B. catarrhalis[*]	44	(11.4)	225	(18.2)	269	(16.6)
H. infl. + *S. pneumoniae*[*]	19	(4.9)	69	(5.5)	88	(5.4)
H. infl. + *B. catarrhalis*[*]	11	(2.8)	40	(3.2)	51	(3.1)
S. pneum. + *B. catarrhalis*[*]	8	(2.1)	53	(4.3)	61	(3.8)
S. pneum. + *H. infl.* + *B. cat.*[*]	15	(3.9)	22	(1.8)	37	(2.3)
P. aeruginosa[*]	20	(5.2)	80	(6.5)	100	(6.2)
various other bacteria	83	(21.5)	252	(20.4)	335	(20.7)
Total positive cultures	386	(100)	1,237	(100)	1,623	(100)

[*]The total percentage for these groups is, hospital out patients 73.3, hospital in patients 73.1 and together 73.1.

specimens was slightly less than that of the organisms from the hospitalized patients. *Streptococcus pneumoniae* has always been sensitive to ampicillin and erythromycin and this situation did not change over the course of the years. However, the degree of susceptibility to cotrimoxazole and doxycycline has shown some variations but, in general, only 6–7% of strains have been resistant to these agents. Owing to a laboratory error in 1983, an unauthorized change was made in the medium used for testing susceptibility to cotrimoxazole, and this was only discovered in 1986. False resistance was found (due to the presence of antagonists in the medium), but the only effects were upon the results with *H. influenzae* (see table 4).

B. catarrhalis is often not truly sensitive to ampicillin because of the relatively frequent occurrence of low-level β-lactamase production [7] and β-lactamase producing strains have risen (with some fluctuations) from zero (in 1979) to approximately 63% of the total in recent years. Nevertheless, despite a constant search, the first β-lactamase producing strain of *B. catarrhalis* was only detected in February 1980. The *B. catarrhalis* strains have remained highly sensitive to doxycycline, erythromycin and cotrimoxazole, with only few exceptions. The occurrence of β-lactamase producing strains of *Haemophilus influenzae* has been relatively constant since 1980 and averaged 2–6% (table 5). However, monthly variations have been observed, from no production at all up to 10% of the strains cultured, and therefore the overall percentages just quoted are indeed only the yearly averages. Beta-lactamase production by *Haemophilus parainfluenzae* was noted in 13% of strains in 1981 but the disappearance of this organism has changed the picture somewhat.

Figure 2 gives an overview of the mean monthly totals of *H. influenzae* and *Branhamella catarrhalis* over the five-year period 1982–1986. Although *H. influenzae* is cultured in almost constant numbers throughout the whole year, the proportion of sputum specimens yielding *Branhamella catarrhalis* shows a

Table 4. - Percentages of bacteria fully sensitive to various antimicrobial agents

Results in:	1982	1983	1984	1985	1986	1987
H. influenzae						
ampicillin	96	95	90	96	97	92
cotrimoxazole	99	64*	83*	83*	41*	95
doxycycline	98	94	96	93	89	88
erythromycin	97	92	96	80	50**	47**
ofloxacin	-	-	-	100	100	100
S. pneumoniae						
ampicillin	98	100	100	100	100	100
cotrimoxazole	93	84	92	97	93	98
doxycycline	95	98	93	97	94	97
erythromycin	100	100	100	100	100	100
ofloxacin	-	-	-	100	100	99
B. catarrhalis						
ampicillin	78	46	49	59	30	37
cotrimoxazole	93	76	97	98	99	99
doxycycline	98	97	99	97	96	85
erythromycin	100	100	99	98	100	100
ofloxacin	-	-	-	100	100	100
P. aeruginosa						
ampicillin	5	2	6	4	7	6
cotrimoxazole	3	10	12	15	0	6
doxycycline	5	0	2	0	7	6
erythromycin	0	0	0	0	0	0
ofloxacin	-	-	-	83	86	79

*: figures not accurate due to antagonists in the test medium; **: new criteria employed for erythromycin from January 1986

Table 5. - ß-lactamase production in respiratory pathogens 1980-1986 as percentages of total isolates of each species

Year:	1980	1981	1982	1983	1984	1985	1986
H. influenzae	4	5	2	4	6	4	6
H. parainfluenzae	13	8	7	*	*	*	*
S. pneumoniae	-	-	-	-	-	-	-
B. catarrhalis	7	38	22	44	51	70	63

*: too few strains cultured.

typical seasonal pattern with deep dips in the summer and high peaks in the winter months.

Table 6 gives an overview of the theoretical chances of failure with the blind use of various oral antimicrobial agents when given in adequate doses to our patient population. The calculations (based on the percentage frequency of occurrence of the various bacteria, alone or in combination, multiplied by the percentage frequency of drug resistance and then divided by 10,000) show a likelihood of failure of 8–29% with cotrimoxazole, 14–35% with doxycycline, 24–45% with erythromycin and 25–45% with the aminopenicillins. The two figures for each antimicrobial agent represent the extreme possibilities for the 'various other bacteria' which cannot be analysed in detail here. The last column in the table shows the corresponding chances of treatment failure with the modern quinolone agent ofloxacin.

Discussion

In the previous ten years the pathogenic bacterial flora from airways-infections in the southern part of The Netherlands has shown quite considerable qualitative and quantitative changes. The most impressive change has been the increase in β-lactamase producing *Branhamella catarrhalis*. In general, *Haemophilus influenzae* has continued to play a predominant role in this district, although the slight increase in resistance to aminopenicillins has led to some problems, especially in general practice. The drugs most frequently used outside hospital were originally ampicillin (now replaced by amoxycillin) and cotrimoxazole, doxycycline or erythromycin, although a 25% failure-rate has often been noted. Cotrimoxazole appears at the present time to yield the best therapeutic results for blind therapy but doxycycline

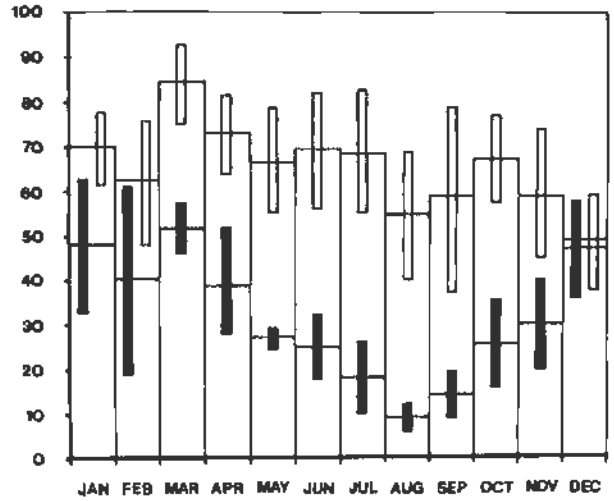

Fig. 2. Mean monthly totals of isolates of *Haemophilus influenzae* (upper blocks) and *Branhamella catarrhalis* (lower blocks) between 1982 and 1986. Vertical bars represent standard deviations from the mean.

may still be useful, as we have shown in a retrospective comparative study in which the results from recent times were analysed and compared with those noted 17 yrs previously [8].

If antimicrobial chemotherapy for any infection is to be successful, then there are only two options available to the clinician. Either a suitable specimen (such as sputum) must be cultured and susceptibility tests carried out on any significant pathogens grown, so allowing precision of antimicrobial therapy, or the treatment must be commenced blindly. Although we do not wish to encourage the use of blind treatment, we recognise that many practitioners (particularly those outside the hospitals) manage their patients in this way. For this reason, we believe it essential that an analysis should be made of the frequency of occurrence of the various infecting organisms in each district, and that this analysis should be combined with an assessment of the susceptibility of the pathogens to a series of commonly used antimicrobial agents. We have found that such calculations of the chances of success (or failure) do, in fact, agrec with reality, even when patients have been treated blindly outside hospital.

Apart from the apparent disappearance of *H. parainfluenzae* which may have been due to improvements in the identification of those *H. influenzae* strains able to store X factor endogenously after growth on the primary medium [5], we do not have any explanation for the extensive changes in the bacterial flora. In any case, selection pressure from the use of certain antimicrobial agents such as (in order of importance) ampicillin, amoxycillin, cotrimoxazole and, to a lesser extent, doxycycline and erythromycin cannot be the explanation for the development of resistance. If this were so, then one would expect that typical strains of *Streptococcus pneumoniae* (which remain highly sensitive to these

Table 6. - Calculated chance of failure with blind use of antimicrobial agents in respiratory infection as of January 1987

Bacterial species	% occurrence	cotrimoxazole % Resistant	cotrimoxazole Chance of failure	doxycycline % Resistant	doxycycline Chance of failure	erythromycin % Resistant	erythromycin Chance of failure	ampi/amoxycillin % Resistant	ampi/amoxycillin Chance of failure	ofloxacin % Resistant	ofloxacin Chance of failure
H. influenzae	25.9	5	0.013	12	0.031	53	0.137	8	0.021	0	0.000
S. pneumoniae	16.0	2	0.003	3	0.005	0	0.000	0	0.000	1	0.002
B.catarrhalis	16.6	1	0.002	15	0.025	0	0.000	63	0.105	0	0.000
H.influ.+S. pneumoniae	5.4	5	0.003	12	0.006	53	0.029	8	0.004	1	0.001
H.influ.+B.catarrhalis	3.1	5	0.003	15	0.005	53	0.016	63	0.020	0	0.000
S.pneum.+B.catarrhalis	3.8	2	0.001	15	0.006	0	0.000	63	0.024	1	0.000
S.pneum.+H.infl.+B.cat.	2.3	2	0.000	15	0.003	0	0.000	63	0.014	1	0.000
P.aeruginosa	6.2	94	0.058	94	0.058	100	0.062	94	0.058	21	0.013
Various other bacteria	20.7+	0	0.000	0	0.000	0	0.000	0	0.000	0	0.000
	20.7+	100	0.207	100	0.207	100	0.207	100	0.207	100	0.207
TOTAL	100.0		0.082		0.139		0.244		0.246		0.016
Depending on resistance of "other bacteria' up to			0.289		0.346		0.451		0.453		0.223

+The two lines of figures represent potential extremes from 100% susceptible to 100% resistant. Chance of failure=[frequency of occurrence (%)xfrequency of drug resistance (%)]/10,000.

agents) would have disappeared. That this is not the case may sometimes be the result of the presence of β-lactamase producing *B. catarrhalis* or *H. influenzae* strains destroying penicillins and thus protecting the pneumococci. At the same time, the inexplicable rise in *Branhamella catarrhalis* in a relatively constant antibiotic climate cannot be explained by any such selection process and is not merely the result of increased awareness in the laboratory. It is very curious that the development of β-lactamase producing *Branhamella catarrhalis* has been described in Western Europe, Great Britain and in the United States almost exactly at the same time and quite independently. Moreover, it is now clear that these organisms are more frequently found in industrialized districts such as Scotland [9], and the USA [10] and that they also appear to be affected by the time of the year. That this phenomenon could be due to the changing degrees of air pollution with oxides of sulphur and nitrogen is a possible hypothesis, but the question must still remain completely open. Although the Gram-negative respiratory pathogens are highly sensitive to the newer quinolone agents such as ofloxacin, only time will tell whether the relative insensitivity of *S. pneumoniae* to these agents [11] causes problems in actual practice.

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RÉSUMÉ: Les résultats des cultures de crachat et les tests de susceptibilitié ont été revus pour les années 1977 à 1986 avec environ 1,700 cultures positives par an. *Haemophilus influenzae* a toujours occupé la première place, ayant apparu dans 65% des spécimens positifs en 1977 et dans 37% des échantillons en 1986. *Streptococcus pneumoniae* occupé la deuxième place, avec une augmentation de 17 à 27% au cours de la même période durant laquelle *Branhamella catarrhalis* est passée de 5 à 26%. Les chances théoriques pour qu'une thérapie à l'aveugle échoue ont été calculées selon la fréquence d'apparition des espèces bactériennes individuelles dans les cultures de crachat et selon le pourcentage de souches résistantes à l'ampicilline, la cotrimoxazole, la deoxycycline, l'érythromycine et l'ofloxacine, avec cependant un plus grand avantage pour l'ofloxacine et la cotrimoxazole. Aucune explication ne pouvait être fournie pour l'augmentation de la β-lactamase produite par *B. catarrhalis*, mais la disparition de *Haemophilus parainfluenzae* aurait pu résulter des améliorations du procédé d'identification.