High frequency of multiresistant respiratory tract pathogens at community level in South India

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Objective: To describe the patterns of antibiotic susceptibility of outpatient strains of *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* in the district of Pondicherry in South India.

Methods: The antibiotic susceptibilities of 94 *S. pneumoniae*, 97 *H. influenzae* and 104 *M. catarrhalis* strains, collected from outpatients with respiratory tract infections, were determined by disk diffusion and Etest.

Results: Resistance or reduced susceptibility to trimethoprim–sulfamethoxazole was found in 67% of *S. pneumoniae*, 53% of *H. influenzae* and 24% of *M. catarrhalis* strains. Thirty-seven per cent of *S. pneumoniae* and 39% of *H. influenzae* strains were resistant or showed reduced susceptibility to tetracycline. Reduced susceptibility to penicillin was found in 6% of *S. pneumoniae* strains. Overall, 10% of *S. pneumoniae* and 38% of *H. influenzae* strains showed reduced susceptibility to ≥3 antibiotics. Comparisons between the antibiotic susceptibility patterns of the Indian strains and a corresponding collection of strains from Sweden indicate that the susceptibility of the native susceptible population is independent of geographic origin.

Conclusions: The findings indicate high consumption of tetracycline and trimethoprim–sulfamethoxazole in the area, which emphasizes the need for surveillance of the pattern of antibiotic susceptibility among respiratory tract pathogens at community level in developing countries and for the implementation of local guidelines for rational use of antibiotics.

Key words: Antibiotic resistance, *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis,* developing countries

INTRODUCTION

Acute respiratory tract infections (ARIs) are major causes of morbidity and mortality in developing countries, especially among children [1]. *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* are common bacterial pathogens in ARI. These pathogens may harbor genes coding for resistance mechanisms which may affect several antibiotics commonly used in the treatment of ARI. Therefore, knowledge about patterns of antibiotic susceptibility is necessary for appropriate use of antibiotics [2,3]. However, in developing countries, such information is often sparse, especially at community level, where most of the antibiotics are prescribed [4–7].

In the few reports that are available from developing countries, a high frequency of resistance to several of the antibiotics that are included in the ninth WHO Model List of Essential Drugs [8] has been documented. For example, in a study performed in Zambia, 23% of outpatient isolates of *S. pneumoniae* collected from children showed resistance to tetracycline [4], and from Pakistan it was reported that 33% of invasive *H. influenzae* isolates collected from children were resistant or showed reduced susceptibility to trimethoprim–sulfamethoxazole [5].

The objective of this study was to describe the patterns of antibiotic susceptibility among important respiratory tract pathogens collected from outpatients

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in the district of Pondicherry in South India. The patterns of antibiotic susceptibility at community level could form the basis for recommendations regarding empirical therapy and for the implementation of local guidelines for rational use of antibiotics. This survey was performed in collaboration with the Department of Microbiology at the Jawaharlal Institute of Medical Education and Research (JIPMER) in Pondicherry, and was part of an Indo-Swedish project in which efforts were also made to facilitate continued local monitoring of antibiotic susceptibility. For this purpose, the project included transfer of microbiological technology and equipment. In addition to the local survey in India, the antibiotic susceptibilities of the strains collected in India were compared with those of a corresponding collection of strains from Sweden.

MATERIALS AND METHODS

Study population in India

The survey was performed in the district of Pondicherry. Of the 450 000 inhabitants in the district, 43% lived in rural areas and 57% in urban areas. Health care was provided by several health centers, private practitioners, and a few hospitals.

Collection of strains in India

Isolates of *S. pneumoniae, H. influenzae* and *M. catarrhalis* were obtained through nasopharyngeal cultures from outpatients with respiratory tract infections attending healthcare facilities in the district. Twelve of 28 health centers located in rural and urban areas and the outpatient department of the General Hospital in Pondicherry city were included in the study to obtain bacterial isolates from patients that would represent the whole population in the district. Nasopharyngeal specimens were collected by specially trained staff until about 100 isolates of each species had been identified. Seventy per cent of the isolates were recovered from children below the age of 16 years. The nasopharyngeal specimens were transported in modified Stuart's medium to the Department of Microbiology at JIPMER Hospital, where primary culture, species identification and susceptibility testing by disk diffusion were performed. The isolates were frozen in fetal calf serum at −70°C, and later transported on dry ice to Sweden for repeated species identification and susceptibility testing by disk diffusion and Etest at the Department of Clinical Microbiology at Växjö Central Hospital.

Strains from Sweden

Results obtained from routine susceptibility testing of consecutive outpatient strains of *S. pneumoniae, H. influenzae* and *M. catarrhalis* at the Department of Clinical Microbiology at Växjö Central Hospital were included for comparison with the strains collected in India. The number of strains tested against each antibiotic is indicated in Figures 1–3.

Primary culture

The nasopharyngeal swab was plated on a chocolate agar plate (blood agar base II (Oxoid, Basingstoke, UK) supplemented with 7% horse blood) and on a double-layered blood agar plate (blood agar base and blood agar base II (Oxoid), supplemented with 6% horse blood). An optochin disk (Oxoid) was placed on the primary streak immediately before incubation in order to provide rapid indication of the presence of pneumococci. The chocolate agar plate was incubated in an atmosphere of 5% CO_2 at 37°C overnight, and the double-layered blood agar plate was incubated anaerobically at 37°C overnight.

Species identification

S. pneumoniae was identified by typical, mucoid or non-mucoid, optochin-sensitive colonies on blood agar or chocolate agar. *H. influenzae* was identified by translucent colonies on chocolate agar and tested for X and V factor dependence. *M. catarrhalis* was identified by non-pigmented oxidase-producing cohesive colonies on chocolate agar.

Susceptibility testing

The disk diffusion method was performed according to standardized procedures [9–11] (antibiotic disks from Oxoid). Plates were inoculated by the swab technique with a bacterial suspension of approximately 10^6 CFU/mL to yield semi-confluent growth. For *H. influenzae* and *M. catarrhalis*, Iso-sensitest Agar (Oxoid) supplemented with 1% hemoglobin (Oxoid) and 1% Iso-Vitalex (BBL, Cockeysville, MD, USA) was used. For *S. pneumoniae*, Iso-sensitest Agar (Oxoid) supplemented with 5% defibrinated horse blood was used. All plates were incubated at 37°C for 16–20 h in an atmosphere of 5% CO_2. *H. influenzae* and *M. catarrhalis* were examined for β-lactamase production by using nitrocefin-based sticks (Oxoid). Minimum inhibitory concentrations (MICs) were determined by the Etest method (AB Biodisk, Solna, Sweden) [12]. MICs of cefotaxime, tetracycline, erythromycin, chloramphenicol and trimethoprim–sulfamethoxazole were determined for all bacterial strains collected in India. In addition, MICs of benzylpenicillin were determined for *S. pneumoniae* strains, and MICs of amoxycillin for β-lactamase-negative strains of *H. influenzae* and *M. catarrhalis*. Zone diameters for different antibiotics (see Figures 1–3), obtained by the disk diffusion method,

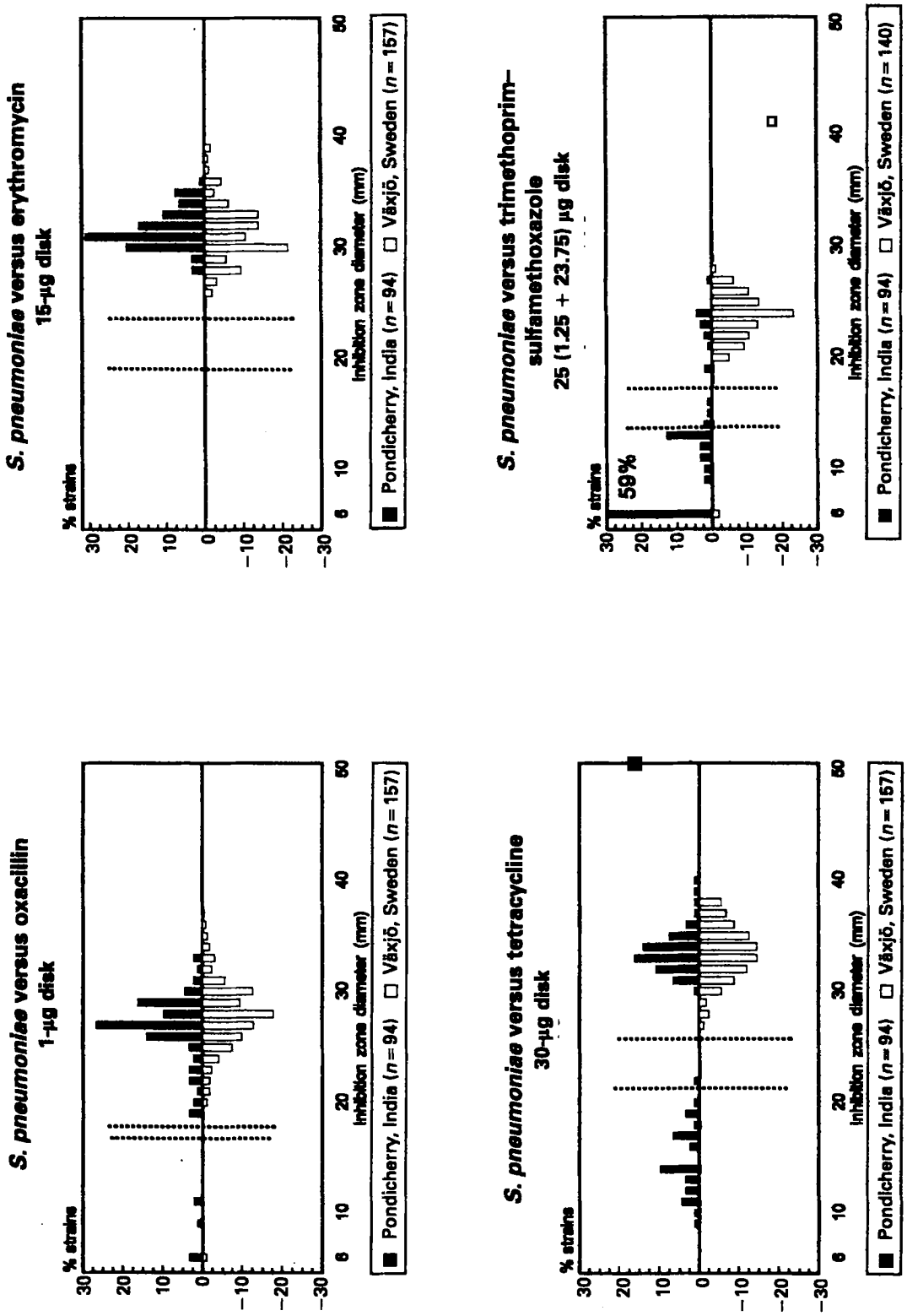

Figure 1 Histograms of antibiotic inhibition zones of *S. pneumoniae* isolates recovered from outpatients with ARI from India (filled bars) and from Sweden (open bars). Zone breakpoints for *S. pneumoniae*, recommended by the SRGA, are indicated by dotted lines.

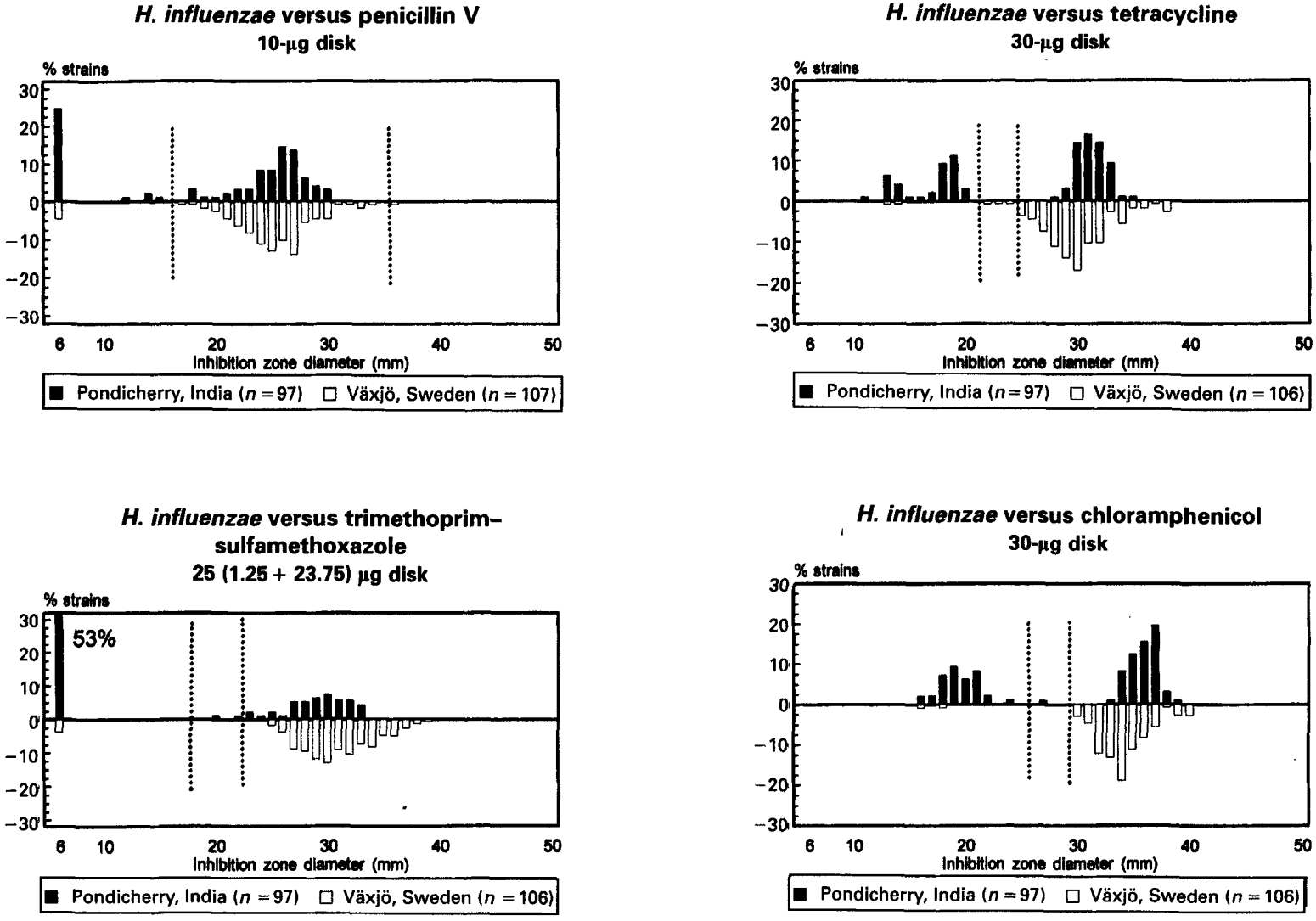

Figure 2 Histograms of antibiotic inhibition zones of *H. influenzae* isolates recovered from outpatients with ARI from India (filled bars) and from Sweden (open bars). Zone breakpoints for *H. influenzae*, recommended by the SRGA, are indicated by dotted lines.

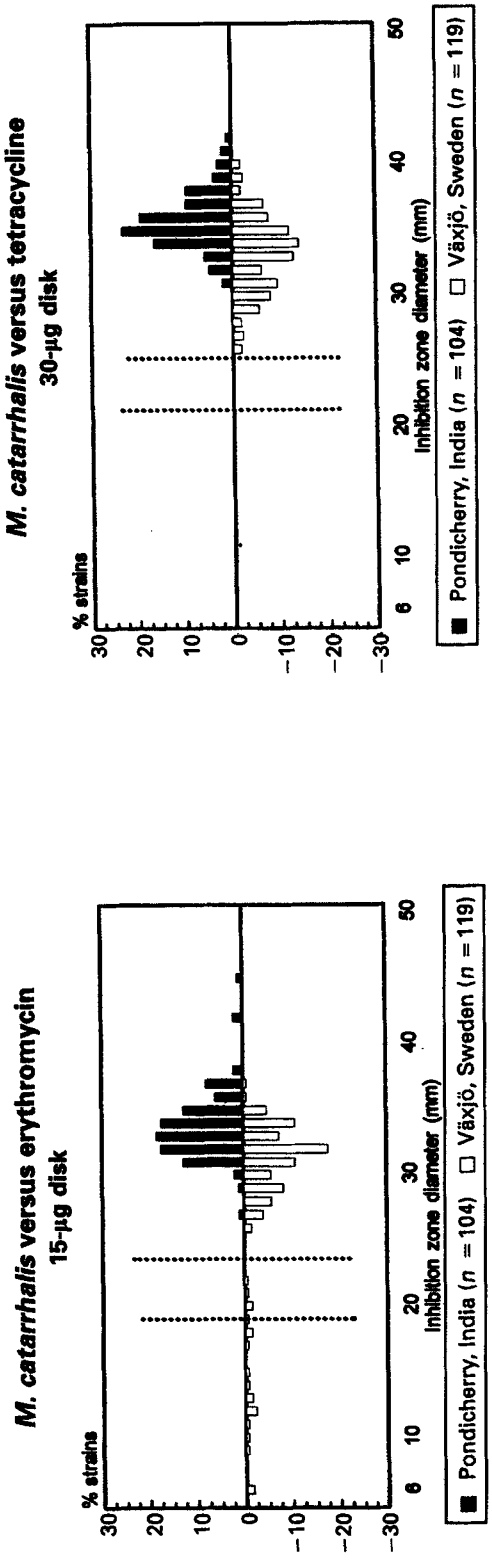

Figure 3 Histograms of antibiotic inhibition zones of *M. catarrhalis* isolates recovered from outpatients with ARI from India (filled bars) and from Sweden (open bars). Zone breakpoints for *M. catarrhalis*, recommended by the SRGA, are indicated by dotted lines.

were determined for the Indian and the Swedish collections of strains. Penicillin resistance in *S. pneumoniae* was detected with an oxacillin 1-µg disk (Oxoid). Control strains for susceptibility testing by Etest and disk diffusion were *S. pneumoniae* ATCC 49619, *H. influenzae* NCTC 8468, and *M. catarrhalis* ATCC 8176.

Definitions of susceptibility and resistance

MIC and zone breakpoints were interpreted according to the Swedish Reference Group for Antibiotics (SRGA) [10,11,13].

RESULTS

Streptococcus pneumoniae

Six per cent (6/94) of the pneumococcal strains from India showed reduced susceptibility to penicillin (MIC ≥0.125 mg/L), and 3% (3/94) showed reduced susceptibility to cefotaxime (MIC ≥0.25 mg/L) (Table 1). Resistance to tetracycline (MIC ≥4 mg/L) and chloramphenicol (MIC ≥16 mg/L) was found in 37% (35/94) and 4% (4/94) of the isolates, respectively. No resistance to erythromycin was found. Fifteen per cent (14/94) of the pneumoccocal isolates showed reduced susceptibility to trimethoprim–sulfamethoxazole (MIC=32 (1.6+30.4) mg/L), and 52% (49/94) were resistant (MIC ≥64 (3.2+60.8) mg/L). Overall, 10% of the pneumococcal isolates were resistant or showed reduced susceptibility to three or more of the tested antibiotics.

Haemophilus influenzae

The proportion of β-lactamase-producing *H. influenzae* strains from India was 29% (28/97). In β-lactamase-negative strains, resistance to amoxycillin (MIC ≥1 mg/L) was 7% (5/69) (Table 2). All 97 isolates of *H. influenzae* were susceptible to cefotaxime (MIC ≤0.064 mg/L). Resistance to tetracycline (MIC ≥4 mg/L), chloramphenicol (MIC ≥4 mg/L) and trimethoprim–sulfamethoxazole (MIC ≥64 (3.2+60.8) mg/L) was frequent, 39% (38/97), 38% (37/97) and 53% (51/97), respectively. Apart from inherently low susceptibility to erythromycin (MIC 1–16 mg/L), overall 38% of *H. influenzae* strains were resistant or showed reduced susceptibility to three or more of the tested antibiotics.

Moraxella catarrhalis

ß-Lactamase production was found in 68% (71/104) of *M. catarrhalis* strains from India. All 33 strains that were ß-lactamase negative were susceptible to amoxycillin (MIC ≤2 mg/L) (Table 3). All 104 isolates of *M. catarrhalis* were susceptible to cefotaxime (MIC ≤8 mg/L), erythromycin (MIC ≤0.5 mg/L) and chloram-

Table 1 Antibiotic susceptibilities of 94 *S. pneumoniae* isolates from Pondicherry district

	Minimum inhibitory concentration (mg/L)[a]														
Antibiotic	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	≥64
Benzylpenicillin	S	13S	53 S	19 S	3 S	1 I	2 I	3 I	I	R	R	R	R	R	R
Cefotaxime	S	10S	51 S	22 S	4 S	4 S	3 I	I	I	R	R	R	R	R	R
Tetracycline	S	S	S	S	S	4 S	45 S	10 S	S	S	R	1 R	1 R	6 R	27 R
Erythromycin	S	S	S	S	19 S	73 S	2 S	S	R	R	R	R	R	R	R
Chloramphenicol	S	S	S	S	S	S	S	1 S	3 S	75 S	9 S	2 S	3 R	1 R	R
Trimethoprim–sulfamethoxazole[b]	S	S	S	S	S	S	S	S	S	1 S	10 S	2 S	18 S	14 I	49 R

[a]MIC breakpoints, recommended by the SRGA, are indicated by S (susceptible), I (intermediately susceptible), and R (resistant). The numerals indicate number of strains.
[b]Trimethoprim/sulfamethoxazole, 1:19 ratio.

Table 2 Antibiotic susceptibilities of 97 *H. influenzae* isolates from Pondicherry district

	Minimum inhibitory concentration (mg/L)[a]														
Antibiotic	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	≥64
Amoxycillin[b]	S	S	S	S	S	S	13 S	51 S	5 R	R	R	R	R	R	R
Cefotaxime	4 S	8 S	53 S	32 S	S	I	I	I	I	R	R	R	R	R	R
Tetracycline	S	S	S	S	S	S	S	S	33 S	26 S	R	8 R	14 R	5 R	11 R
Erythromycin	I	I	I	I	I	I	I	I	2 I	26 I	51 I	17 I	1 I	I	I
Chloramphenicol	S	S	S	S	S	S	S	28 S	32 S	S	3 R	27 R	6 R	1 R	R
Trimethoprim–sulfamethoxazole[c]	S	S	S	S	S	S	S	10 S	18 S	15 S	3 S	S	S	I	51 R

[a]MIC breakpoints, recommended by the SRGA, are indicated by S (susceptible), I (intermediately susceptible), and R (resistant). The numerals indicate number of strains.
[b]MIC values for β-lactamase-negative strains.
[c]Trimethoprim/sulfamethoxazole, 1:19 ratio.

Table 3 Antibiotic susceptibilities of 104 *M. catarrhalis* isolates from Pondicherry district

	Minimum inhibitory concentration (mg/L)[a]														
Antibiotic	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	≥64
Amoxycillin[b]	S	S	7 S	10 S	12 S	4 S	S	S	S	S	I	I	R	R	R
Cefotaxime	S	S	S	6 S	15 S	28 S	38 S	15 S	2 S	S	S	S	R	R	R
Tetracycline	S	S	S	S	S	1 S	5 S	50 S	47 S	S	1 R	R	R	R	R
Erythromycin	S	S	S	S	3 S	19 S	50 S	32 S	I	I	I	R	R	R	R
Chloramphenicol	S	S	S	1 S	S	1 S	11 S	88 S	3 S	S	S	S	R	R	R
Trimethoprim–sulfamethoxazole[c]	S	S	S	S	S	S	S	S	S	1 S	2 S	16 S	60 S	16 I	9 R

[a]MIC breakpoints, recommended by the SRGA, are indicated by S (susceptible), I (intermediately susceptible), and R (resistant). The numerals indicate number of strains.
[b]MIC values for β-lactamase-negative strains.
[c]Trimethoprim/sulfamethoxazole, 1:19 ratio.

phenicol (MIC ≤8 mg/L). Resistance to tetracycline (MIC ≥4 mg/L) was found in 1% (1/104). Fifteen per cent (16/104) showed reduced susceptibility to trimethoprim–sulfamethoxazole (MIC=32 (1.6+30.4) mg/L), and 9% (9/104) were resistant (MIC ≥64 (3.2 + 60.8) mg/L).

Comparison of Indian and Swedish strains

The antibiotic susceptibility patterns of the respiratory tract pathogens from India were compared with those of a corresponding collection of strains from Sweden by disk diffusion. The results are presented in histograms of zone inhibition (Figures 1–3). For each of the

tested antibiotic–species combinations, the native susceptible populations showed similar distributions and locations in the zone diameter histograms, independent of geographic origin (Figures 1–3). However, subpopulations with acquired antibiotic resistance varied widely between the different geographic origins. For example, *S. pneumoniae* strains from India showed large subpopulations that were resistant to tetracycline (≤21 mm) and trimethoprim–sulfamethoxazole (≤13 mm), while such subpopulations were not found in the Swedish collection (Figure 1). Indeed, for trimethoprim–sulfamethoxazole, the native susceptible population of *S. pneumoniae* from India was very small and had been replaced by a large resistant population.

The frequency of β-lactamase-producing *H. influenzae* was 29% in strains from India and 5% in strains from Sweden. The Indian collection of *H. influenzae* showed large subpopulations resistant to tetracycline (≤21 mm), trimethoprim–sulfamethoxazole (≤18 mm), and chloramphenicol (≤26 mm). Corresponding subpopulations in the Swedish collection were lacking or were very small (Figure 2).

β-Lactamase was produced in 68% of *M. catarrhalis* strains from India and in 76% of strains from Sweden. The zone diameter histogram for tetracycline showed a similar pattern for *M. catarrhalis* strains from India and from Sweden, while for erythromycin, scattered isolates representing a resistant subpopulation (≤19 mm) were seen in the Swedish collection (Figure 3).

DISCUSSION

In India, as in many other countries, information about antibiotic resistance patterns is incomplete [14]. In this report we describe the antibiotic resistance patterns of *S. pneumoniae, H. influenzae* and *M. catarrhalis* collected from outpatients in the district of Pondicherry in southern India. MICs were determined for a number of antibiotics (for oral administration) that were available and prescribed in the district, and, in addition, for benzylpenicillin and cefotaxime. All of the tested antibiotics are included in the ninth WHO Model List of Essential Drugs [8], except for cefotaxime, which was chosen as a representative of the third-generation cephalosporins and a drug that can be used for treatment of serious infections with *S. pneumoniae* and *H. influenzae*.

In *S. pneumoniae* and *H. influenzae*, high proportions of resistance to tetracycline, 37% and 39% respectively, and trimethoprim–sulfamethoxazole, 67% and 53% respectively, were documented. Since these two species showed the same degree of resistance, a high consumption of tetracycline and trimethoprim–sulfamethoxazole in the district is a more likely explanation than clonal spread of resistant strains. The proportion of *S. pneumoniae* strains with reduced susceptibility to penicillin was 6%, but no isolate was detected with high-level resistance (MIC ≥2 mg/L). Resistance to chloramphenicol was found in 38% of *H. influenzae* strains. Altogether, 10% of *S. pneumoniae* and 38% of *H. influenzae* strains were resistant or showed reduced susceptibility to three or more unrelated antibiotics. In this study, the documented proportion of respiratory tract pathogens with resistance or reduced susceptibility to antibiotics commonly used in the treatment of ARI was higher than that reported from other developing countries [4–7].

The MIC values for erythromycin in *H. influenzae* are presented in this report to allow the reader to compare them with results from other studies. Resistance mechanisms to erythromycin (or other macrolide antibiotics) have yet to be described. Wild-type *H. influenzae* strains are considered to be only marginally susceptible to macrolides and, hence, the SRGA recommends that all *H. influenzae* strains are reported as intermediately susceptible [11]. Thus, erythromycin is not regarded as a relevant drug for treatment of infections caused by *H. influenzae.*

The high proportion of multiresistant strains of *H. influenzae* was particularly troubling. Twenty-eight of the 97 isolates (28%) showed resistance or reduced susceptibility to all of the five oral antibiotics (tetracycline, erythromycin, trimethoprim–sulfamethoxazole, amoxycillin and chloramphenicol) that were commonly used for treatment of ARI in the Pondicherry district. Among strains of *M. catarrhalis*, resistance or reduced susceptibility to trimethoprim–sulfamethoxazole was prevalent (24%). The high frequency of resistance in *M. catarrhalis* to trimethoprim–sulfamethoxazole may reflect high usage of this drug in the area.

The antibiotic susceptibility patterns of the respiratory tract pathogens from the district of Pondicherry documented in this survey may serve as a basis for recommendations for empirical therapy of outpatients with ARI and for implementation of local guidelines for appropriate use of antibiotics. However, factors such as limitations of antibiotic supplies and difficulties in performing routine cultures for susceptibility testing to adjust antibiotic treatment should be considered when formulating such recommendations. Since *S. pneumoniae* is responsible for the majority of bacterial respiratory tract infections [15], and most isolates (94%) of *S. pneumoniae* from Pondicherry were susceptible to penicillin, this antibiotic seems to be a relevant choice as first-line drug for treatment of bacterial ARI. The high frequency of resistance to trimethoprim–

sulfamethoxazole and tetracycline in *S. pneumoniae* and *H. influenzae* indicates high consumption of these two drugs. Restricted use of these two drugs may decrease the frequency of resistant strains.

The antibiotic susceptibility patterns of the pathogens of different geographic origins, from India and Sweden, were compared by disk diffusion. To reduce bias due to variations in media, laboratory techniques etc., the two collections of strains were tested under the same conditions in one laboratory (Växjö, Sweden). The results were presented in histograms of zone inhibition (Figures 1–3). This made it possible to identify and compare the distributions and locations in the histograms of wild-type strains and subpopulations with acquired antibiotic resistance. Most interestingly, the wild-type strains from India and from Sweden showed the same distribution and location in the histograms. This indicates that antibiotic susceptibility of native bacterial populations is independent of geographic origin.

In summary, we have documented a high frequency of multiresistant respiratory tract pathogens at community level in the district of Pondicherry in South India. In addition, the resistance patterns of *S. pneumoniae* and *H. influenzae* indicate a high consumption of tetracycline and trimethoprim–sulfamethoxazole in the area. The findings in this study emphasize the need for surveillance of the patterns of antibiotic susceptibility among important respiratory tract pathogens at community level in developing countries, both as a basis for empirical therapy and for the implementation of local guidelines for rational use of antibiotics. Moreover, the comparisons of the strains collected in India and in Sweden indicate that the antibiotic susceptibility of wild-type strains is independent of geographic origin.

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