Changes in antibiotic resistance rates of invasive 
*Haemophilus influenzae* isolates in England and 
Wales over the last 20 years

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**Objectives:** The aim of this study was to determine trends in antibiotic resistance profiles of invasive clinical *Haemophilus influenzae* isolates over the last 20 years.

**Methods:** Microbiology laboratories throughout England and Wales regularly submit invasive *H. influenzae* isolates to the Health Protection Agency for serotyping and antimicrobial susceptibility testing. Antimicrobial resistance was defined using the British Society for Antimicrobial Chemotherapy criteria (http://bsac.org.uk). All *H. influenzae* isolates from blood and cerebrospinal fluid (CSF) cultures between January 1985 and December 2004 were included.

**Results:** Over the 20 year study period, 6805 *H. influenzae* isolates from blood (*n* = 4932, 72.5%) and CSF (*n* = 1873, 27.5%) were obtained. Over half the isolates (3736/6805, 54.9%) were identified as Hib, 38.9% (*n* = 2644) as non-capsulated and 6.2% (*n* = 425) as other capsulated serotypes. Resistance to ampicillin was highest at 16.2%, followed by trimethoprim (7.7%), tetracycline (1.8%) and chloramphenicol (1.2%). All isolates were susceptible to cefotaxime and only four (0.06%) were resistant to rifampicin. When analysing trends, chloramphenicol and tetracycline resistance rates have remained low since the late 1980s. Ampicillin resistance increased slowly until the mid-1990s and then gradually declined to its lowest rate of 11.6% in 2004. Resistance to trimethoprim initially fell from 10% in 1985 to 1.8% in 1989, but has continued to rise since then to 11.9% in 2004. In a logistic regression model, year of isolate (*P* < 0.001), non-capsulated *H. influenzae* (*P* < 0.001) and younger age (*P* = 0.004) remained independently associated with trimethoprim resistance.

**Conclusions:** Rifampicin and cefotaxime remain highly effective against all invasive *H. influenzae* isolates. Resistance to ampicillin, chloramphenicol and tetracycline has declined over the past 10 years, but trimethoprim resistance continues to increase rapidly. This finding requires further study but may reflect increased use of trimethoprim in the childhood population.

**Keywords:** *Haemophilus influenzae* serotype b, trimethoprim, antimicrobial resistance surveillance

**Introduction**

*Haemophilus influenzae* can cause life-threatening invasive disease in adults and children, including meningitis, septic shock and epiglottitis.1 Early empirical treatment with effective antibiotics is vital in order to prevent serious complications and death. Ampicillin is no longer recommended because of widespread resistance, and although cephalosporins are highly effective against *H. influenzae*, there are already concerns regarding their use in empirical treatment because of increasing resistance.2–4 Continued surveillance of antimicrobial susceptibility is important in order to monitor resistance trends and identify emerging resistance early. The objective of this study was to determine changes in antibiotic resistance profiles of invasive clinical *H. influenzae* isolates between 1985 and 2004, with the aim of identifying long-term trends and recommending appropriate antibiotics for treatment and prophylaxis.

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Antibiotic resistance in invasive *Haemophilus influenzae* isolates

Methods

The Health Protection Agency (HPA) Centre for Infections receives *H. influenzae* isolates and laboratory reports of invasive *H. influenzae* infections from nearly 400 hospitals and public health laboratories. Between 1985 and 1990, *H. influenzae* isolates were submitted on a voluntary basis for identity confirmation, serotyping and antimicrobial susceptibility testing. In September 1990, an enhanced surveillance was initiated in five English regions (East Anglia, Northern, North West, South West and Oxford) and Wales, and extended nationally in 1995 following the rapid decline in Hib incidence resulting from national immunization. Between 1992 and 2000, enhanced surveillance was complemented by a national clinical paediatric reporting scheme through the British Paediatric Surveillance Unit. All reports of *H. influenzae* isolated from a sterile site were followed-up by contacting the microbiologist and requesting referral of the isolate to the HPA Haemophilus Reference Unit if this had not been already done. Serotyping was performed using standard slide agglutination and confirmed by PCR-based capsular genotyping.

Antimicrobial susceptibility testing was carried out using a standard disc sensitivity method according to the British Society for Antimicrobial Chemotherapy (BSAC) guidelines. Strains were plated out on Iso-Sensitest agar, supplemented with 5% lysed equine blood and 0.2% NAD solution. The following disc concentrations were used: ampicillin 2 μg, trimethoprim 2.5 μg, chloramphenicol 10 μg, tetracycline 10 μg, cefotaxime 30 μg and rifampicin 2 μg. Strains were tested for β-lactamase activity using Intralactam Strips (Mast Laboratories Ltd). All cases were entered into a single *Database* database, reconciled and de-duplicated regularly and analysed using Stata 9.0 (www.stata.com). Proportions were compared using the χ² test. Risk factors for trimethoprim resistance were assessed using backward stepwise logistic regression.

Results

Of the 6805 blood (n = 4932, 72.5%) and cerebrospinal fluid (CSF) (n = 1873, 27.5%) isolates from patients with invasive *H. influenzae* infection between 1985 and 2004, 54.9% (n = 3736) were identified as Hib, 6.2% (n = 425) as other capsulated strains and 38.9% (n = 2644) as non-capsulated strains. CSF isolates were mostly Hib (83.0%) followed by non-capsulated (14.0%) and other capsulated serotypes (3.0%), compared with 44.2%, 48.3% and 7.5%, respectively, among blood culture isolates. Half the samples (n = 3398, 49.9%) were from children <16 years, of which 38.9% were from CSF and 76.4% were due to Hib compared with 12.2% and 27.5% in adults, respectively.

Figure 1 depicts the antibiotic resistance rates for invasive clinical *H. influenzae* isolates between 1985 and 2004. Resistance to ampicillin was most common (16.2%), followed by trimethoprim (7.7%), tetracycline (1.8%) and chloramphenicol (1.2%) (Table 1). All isolates were susceptible to cefotaxime and only four (0.06%) were resistant to rifampicin (two non-capsulated, one Hib and one serotype e). Among ampicillin-resistant isolates, β-lactamase production was detected in 97.5% of the 1047 ampicillin-resistant and none of the 5408 ampicillin-susceptible isolates tested. β-Lactamase-negative, ampicillin-resistant (BLNAR) isolates first appeared in 1991, peaked at 8.5% in 1998 and then remained <5% in subsequent years. BLNAR accounted for 0.7% of the 609 Hib isolates, none of 27 other capsulated and 5.4% of the 411 non-capsulated isolates.

Comparison of antibiotic resistances between two 10 year time periods (1985–94 and 1995–2004) showed downward trends for ampicillin (427/2488, 17.2% → 624/4002, 15.6%; χ² = 2.8; P = 0.095), chloramphenicol (32/2482, 1.3% → 44/3985, 1.1%; χ² = 0.45; P = 0.50) and tetracycline (53/2449, 2.2% → 62/3978, 1.6%; χ² = 3.16; P = 0.075), although these were not statistically significant. For ampicillin, the decline in resistance rates was statistically significant after 1998 (χ² for trend = 16.1, P = 0.013). In contrast, trimethoprim resistance increased 3-fold from 3.6% (88/2468 isolates) to 10.2% (407/3988 isolates, χ² = 94.9, P < 0.0001). This increase occurred in both adult (26/584, 4.5% → 224/2337, 9.6%; χ² = 15.7; P < 0.0001) and paediatric (60/1813, 3.3% → 172/1543, 11.1%; χ² = 79.6; P < 0.0001) isolates, blood (50/1144, 4.4% → 374/3597, 10.4%; χ² = 38.7; P < 0.0001) and CSF (38/1324, 2.9% → 33/391, 8.4%; χ² = 23.6; P < 0.0001) isolates, and in Hib (57/2037, 2.8% → 109/1413, 7.7%; χ² = 44.0; P < 0.0001), other capsulated (0/57, 0.0% → 27/346, 7.8%; χ² = 4.8; P = 0.029) and non-capsulated (31/374, 8.3% → 271/2229, 12.2%; χ² = 4.7; P = 0.031) strains. In a logistic regression model, the year of isolate

![Diagram](https://example.com/diagram.png)

Figure 1. Changes in antibiotic resistance rates for *H. influenzae* over 20 years. All isolates were susceptible to cefotaxime and only four (0.06%) were resistant to rifampicin.
Antibiotic resistance rates for clinical *H. influenzae* isolates from 11 countries reported BLNAR rates of 8.8% in 1997/98, 9.6% in 2002/03 and 8.8% in 2004/05, compared with 8.7%, 4.7% and 0% for the same time periods in other European and North American countries, although different methods of antibiotic susceptibility testing have been used in these studies.\textsuperscript{2,3} BLNAR rates are also much lower than those reported elsewhere—one European study of clinical *H. influenzae* isolates from 13 countries,\textsuperscript{10} used in these studies.\textsuperscript{2} The low resistance rates for cephalosporin, rifampicin and tetracycline along with a downward trend in ampicillin resistance since 1998 is also in contrast to reports from other countries where resistance to commonly prescribed antibiotics is increasing rapidly.\textsuperscript{2,4} One reason for this decline may be the recent drive in the UK to reduce antibiotic prescription in the community (up to 50% reduction between 1993 and 1999).\textsuperscript{8}

In contrast, trimethoprim resistance has increased significantly since the early 1990s, although rates in our study are still significantly lower than reported in other countries.\textsuperscript{10} A recent study involving 2712 *H. influenzae* isolates from 13 countries,
for example, reported that 34.5% of the isolates were resistant to trimethoprim/sulfamethoxazole. The increase in trimethoprim resistance in our study was associated with younger age, and the timing of this increase coincides with guidance published by the Royal College of Physicians in the early 1990s on the management of urinary tract infections in children. These guidelines recommend prolonged courses of low-dose trimethoprim until radiological investigations are complete. Children with renal tract anomalies (e.g. vesico-ureteric reflux) were then advised to continue trimethoprim prophylaxis for several years. These guidelines have therefore resulted in a significant proportion of the childhood population becoming exposed to trimethoprim. The findings in this study raise the possibility that this exposure has resulted in increased trimethoprim resistance among frequently carried bacteria, such as *H. influenzae*.

Our results have important clinical implications. First, they support the use of third-generation cephalosporins for the empirical treatment of serious bacterial infections. Secondly, rifampicin remains the prophylactic agent of choice for the eradication of *H. influenzae* among carriers and vulnerable contacts. Thirdly, although the ampicillin resistance rate of 11.6% in 2004 would still be considered too high for empirical treatment, this antibiotic provides a safe and effective therapeutic option if the strain is reported to be ampicillin-susceptible. Finally, the dramatic increase in trimethoprim resistance is of concern and is perhaps a timely reminder of the need for the judicious use of antibiotics.

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**Transparency declarations**

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**References**