Introduction

Increasing problems with antimicrobial resistance have led to concern that we may be returning to a time when antibiotics are no longer effective treatments for common infectious diseases. This concern has resulted in a number of high profile reports such as those from the House of Lords Select Committee on Science and Technology,1 and the US Office of Technology Assessment,2 as well as articles in the lay press.

The impact of antibiotic resistance on hospitalized patients has received considerable emphasis, but antibiotic resistance may also have an impact in the community. Patients infected with antibiotic-resistant bacteria in the community are more likely to require hospitalization, have a longer hospital stay and are more likely to die than those infected with sensitive strains.3 A recent report from the Standing Medical Advisory Committee of the Department of Health4 recommends that national guidelines for use of antibiotics in the community should be developed to ensure that the best practice in antimicrobial prescribing becomes routine practice. Ideally, guidelines would take...

Carriage of antibiotic-resistant bacteria by healthy children

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into account the prevalence of antibiotic resistance in the population to be treated. At the present time there is no information on the frequency of antibiotic-resistant bacteria causing infection in patients treated with antibiotics in the community. Antibiotics are frequently prescribed for respiratory tract infections and a laboratory diagnosis is not made in the majority of these patients.

The purpose of this study was to estimate the frequency of isolation of antibiotic-resistant bacteria in mouthwashes and stools from healthy children in Avon.

**Materials and methods**

The Avon Longitudinal Study of Pregnancy and Child-ood (ALSPAC) has as its overall aim the identification of ways in which the health and development of children can be optimized. ALSPAC (http://www.ich.bristol.ac.uk/alspac.html) has followed over 14 000 children enrolled from early pregnancy between 1 April 1991 and 31 December 1992. At the age of 7–8 years all 14 000 children were invited to attend a follow-up clinic for a range of physical and psychological tests. A research nurse was recruited to work in the follow-up clinic and to collect samples required for this antibiotic resistance study. Ethical approval for this study was provided by the United Bristol Healthcare Trust Ethics Committee.

Stool samples were requested and mouthwashes collected from 539 unselected children attending the ALSPAC 7 year follow-up clinic between December 1998 and May 1999. Mouthwashes were collected by asking children to wash 10 mL of sterile water about their mouths and spit the water into a sterile plastic receptacle. Preliminary investigations demonstrated that the mouthwashing technique employed yielded similar rates of recovery of *Staphylococcus aureus* to paired swabs of the anterior nares in the study population. Carers were given a stool collection pack and a stamped addressed box for the return of the stool samples.

Information on the antibiotic consumption of 227 of the subjects was sought by two methods. First, during clinic visits adults accompanying children were asked for details of antibiotic courses taken by the child in the previous year. Secondly, these adults were asked to deliver a questionnaire to the child's general practitioner (GP) requesting details of antibiotic courses prescribed during the lifetime of the child.

Selected antibiotic-resistant isolates were characterized further. Chloramphenicol and ceftazidime MICs were determined for isolates from chloramphenicol and ceftazidime-containing plates, respectively, using Etest strips (AB Biodisk, Solna, Sweden). These bacteria were also identified to species level using API 20E and 20NE strips (bioMérieux SA, Marcy l’Étoile, France) and (where API profiles were ambiguous) 16S rRNA gene sequencing. Gram-positive cocci growing on agar plates containing vancomycin, nalidixic acid and aztreonam or vancomycin and ceftazidime, were confirmed as *Enterococcus* spp. by aesculin hydrolysis and the PYR test.

Analytical isoelectric focusing

Crude bacterial cell extracts were examined by isoelectric focusing (IEF) as described by Matthew et al. Filter
application tabs were used to load all samples on to IEF gels. Analytical IEF was carried out at 15 W for 2 h on Ampholine PAG plates, pH 3.5–9.5 (Pharmacia LKB, Milton Keynes, UK), which were used in accordance with the manufacturer’s instructions. β-Lactamase bands were visualized with nitrocefin. Isoelectric points (pIs) were estimated by comparison with reference proteins, using a pH 4.7–10.6 calibration kit (BDH Chemicals Ltd, Poole, UK). To facilitate the identification of the β-lactamases produced by the Gram-negative bacteria, the IEF samples and gels were treated as described by Payne et al. Escherichia coli containing TEM-1 and SHV-1 were used as controls and reference points when assessing the pIs.

### Bacterial mating experiments

Enteric Gram-negative isolates demonstrating resistance to more than one antibiotic were mated with E. coli UB1832 (non-lactose rifampicin-resistant strain) for 4 h on solid medium. The bacterial matings were streaked on to MacConkey medium containing rifampicin only. Colonies were then tooth-picked on to MacConkey plates containing single or combinations of antibiotics. Frequencies of the bacterial matings were assessed by determining the ratio of the number of transconjugants to the total number of the recipients mated.

### Results

#### Antibiotic consumption

Requests for antibiotic prescription records were returned for 105 (46%) of 227 children for whom forms were issued within 2 months of the end of the study. The majority had received multiple prescriptions during their lives (mean 5.7), with ampicillin/amoxycillin comprising 342/587 (58%) of courses. Other antibiotics constituting 5% of prescriptions or more were erythromycin (12%), penicillin (10%), flucloxacillin (6%) and topical chloramphenicol (for conjunctivitis) (5%). Only seven subjects (6.7%) had never received an antibiotic prescription. In the year preceding attendance at the clinic, 40 (38%) of 105 patients had received antibiotic prescriptions. None of the 105 children had received chloramphenicol, ciprofloxacin or extended spectrum cephalosporins in the previous year.

Information on antibiotic consumption retrieved from forms completed by questioning accompanying adults was not in agreement with GP returns for many subjects. Most notably, of the 40 children reported by GPs to have received prescriptions in the previous year, 24 (60%) were reported to have received no antibiotics by carers. In contrast, only five children reported to have received antibiotics by carers were reported to have not received antibiotics according to their GPs.

### Resistant isolates from mouthwashes

S. aureus was isolated from mouthwashes from 200 (37.1%) of 539 children sampled. The frequency of isolation of S. aureus from mouthwashes in this study (37%) was similar to the frequency expected from anterior nares sampling and from previous studies of mouth samples from children. This method of sampling was well tolerated by the children in this study. Six (3%) of the 200 isolates were resistant to chloramphenicol, and six (3%) were tetracycline resistant. Twelve strains (6%) were erythromycin resistant. Four (2%) of the 200 isolates were methicillin resistant. During the initial period of the study samples were not examined for the presence of Haemophilus spp. or B. catarrhalis, so that 513 samples were examined for the presence of Haemophilus spp. and 450 samples for Branhamella spp. Haemophilus spp. were isolated from 369 (72%) of 513 samples; 63 (17%) were ampicillin resistant, 49 (13.3%) were erythromycin resistant and seven (1.9%) were tetracycline resistant. B. catarrhalis was isolated from 333 (74%) of 450 samples. Twenty-eight (8.4%) strains were erythromycin resistant and 14 (4.2%) were tetracycline resistant. Only two isolates (0.6%) were ampicillin resistant. Group A β-haemolytic streptococci were isolated from 17 of 507 children sampled. None of these isolates were erythromycin resistant and one (5.9%) was tetracycline resistant.

### Resistant isolates from faecal samples

Stool samples were returned from 335 (62%) of 539 of children. Gram-negative bacilli were isolated on ampicillin-containing agar (4 mg/L) from 299 (89%) and on ciprofloxacin-containing agar (1 mg/L) from six (1.8%) of these samples. Thirty-six (11%) stool samples yielded Gram-negative bacilli with high-level resistance to chloramphenicol (MIC > 128 mg/L). There was no statistical correlation between prescriptions for chloramphenicol at any time and the presence of chloramphenicol-resistant Gram-negative bacilli in the stools. Twenty-seven (75%) of 36 isolates also possessed high-level resistance to ampicillin (MIC > 128 mg/L) and were also spectinomycin and streptomycin resistant (MIC > 50 mg/L). This resistance profile is typical of strains harbouring transposons of the Tn21 family, suggesting that the resistance-encoding genes may well be linked and highly transmissible. Preliminary genetic characterization of three of these isolates has confirmed that cotransfer of chloramphenicol, ampicillin and streptomycin resistance occurs under in vitro conditions at frequencies as high as 1/1000. Data on antibiotic consumption derived from carers was available from six children from whom high-level ampicillin- and chloramphenicol-resistant E. coli were isolated. Only one child had received antibiotics in the previous year (amoxycillin) and none had been hospitalized. Four children had siblings who had received antibiotics in the previous year.
Isolates demonstrating resistance to the third-generation cephalosporin ceftazidime were recovered from 17 subjects (3.2%). The identifications and MICs of ceftazidime for the isolates are shown in the Table. Examination of the molecular basis for this resistance showed that six (35%) of 17 isolates possessed ESβ-L of the TEM and SHV variety, as determined by clavulanic acid inhibition and IEF. These strains were Acinetobacter baumannii (1), Klebsiella oxytoca (2), Commonomonas testosteroni (3) and Hafnia alvei (1). Questionnaire data were collected on seven of the children carrying ceftazidime-resistant isolates and this indicated that only one had been exposed to a β-lactam antibiotic (co-amoxiclav) within the previous year and none had been hospitalized within this period.

**Discussion**

Following the introduction of a new class of antibiotic the proportion of isolates of a bacterial species that are resistant increases along a sigmoid distribution. There are many reports in the medical literature that have drawn attention to high rates of respiratory and gastrointestinal infection, high rates of use of antibiotics and outbreaks of infection with antibiotic-resistant bacteria in child care centres. Particular emphasis has been given to the spread of antibiotic-resistant strains of Streptococcus pneumoniae, methicillin-resistant strains of S. aureus and the impact of child care attendance on the spread of antibiotic-resistant bacteria to household contacts of playgroup attendees. These concerns have led several authors to suggest that there should be efforts to reduce antibiotic usage in children undergoing child care to limit the spread of resistant bacteria. By contrast, there is very little information on the frequency of carriage of antibiotic-resistant bacteria in healthy children in the community outside a child care setting. A recent report from Switzerland suggested that the frequency of isolation of resistant bacteria was lower in children in the community than that reported from institutional settings such as child care centres. In this study we have demonstrated that healthy 7-year-old children may carry strains of S. aureus, Haemophilus spp., B. catarrhalis, group A β-haemolytic streptococci and E. coli with acquired resistance to antibiotics to which they have not been exposed. These antibiotics include ceftazidime, chloramphenicol and tetracycline. Perhaps unsurprisingly, carers had poor recollection of antibiotic prescribing to children in this study, when answers were compared with GP written records. The GP records also probably do not include all relevant prescribing information because of poor recording or prescribing by other health care providers. Systemic chloramphenicol or tetracycline are very rarely prescribed to children in the UK and ceftazidime is only prescribed for iv administration to children with specific conditions such as cystic fibrosis, so although the antibiotic records are incomplete, exposure to tetracycline, ceftazidime or chloramphenicol is probably a very rare event in this population.

<table>
<thead>
<tr>
<th>Species identification</th>
<th>MICa</th>
<th>β-Lactamase IEFb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>&gt;32</td>
<td>6.2, 7.4, 8.1</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>&gt;32</td>
<td>5.3</td>
</tr>
<tr>
<td>Ochrobactrum anthropi</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td>Enterobacter cloaceae</td>
<td>&gt;32</td>
<td>7.8</td>
</tr>
<tr>
<td>H. alvei</td>
<td>&gt;32</td>
<td>7.5, 8.0, 8.4</td>
</tr>
<tr>
<td>H. alvei</td>
<td>&gt;32</td>
<td>7.8</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>&gt;32</td>
<td>7.5/8.0</td>
</tr>
<tr>
<td>Flavobacterium oryzihabitans</td>
<td>4</td>
<td>9.0</td>
</tr>
<tr>
<td>O. anthropi</td>
<td>2</td>
<td>7.8</td>
</tr>
<tr>
<td>C. testosteroni</td>
<td>2 (ESβ/L)</td>
<td>5.3</td>
</tr>
<tr>
<td>C. testosteroni</td>
<td>4 (ESβ/L)</td>
<td>7.5/8.0</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>8 (ESβ/L)</td>
<td>7.4</td>
</tr>
<tr>
<td>C. testosteroni</td>
<td>32</td>
<td>ND</td>
</tr>
<tr>
<td>H. alvei</td>
<td>4 (ESβ/L)</td>
<td>ND</td>
</tr>
<tr>
<td>C. testosteroni</td>
<td>4 (ESβ/L)</td>
<td>ND</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>&gt;32 (ESβ/L)</td>
<td>7.6</td>
</tr>
<tr>
<td>E. cloaceae</td>
<td>32</td>
<td>8.3</td>
</tr>
</tbody>
</table>

*MIC of ceftazidime in mg/L.  
βpI of enzyme(s).  
ND, not determined.
Antibiotic resistance may be co-selected by exposure to other antibiotics used in children or may be acquired from family members, pets, other children and food. A recent paper from the Welsh Antibiotic Study Group demonstrated an association between ampicillin and trimethoprim resistance in urinary isolates. The authors suggested that there may be co-selection of resistance to ampicillin and trimethoprim because the genes determining resistance are linked. We have demonstrated that chloramphenicol and ampicillin resistance are linked in isolates of E. coli from healthy children, so that use of ampicillin may be selecting for chloramphenicol resistance in enteric E. coli. An additional factor that facilitates the spread of antibiotic resistance is the presence of transferable genetic elements that allow antibiotic resistance to spread between bacteria even of taxonomically distant species. In this study, resistance in faecal E. coli to ampicillin and chloramphenicol was transmissible between bacteria. In a proportion of ceftazidime-resistant isolates there was evidence of ES/IL coding for resistance. These enzymes are also frequently carried on transmissible elements. Transmissible genetic elements allow antibiotic resistance genes to spread both to commensal bacteria and to strains that cause disease.

Healthy children may acquire bacteria resistant to antibiotics to which they have not been exposed. The levels of resistance illustrate the extent to which antibiotic-resistant bacteria are circulating within the healthy childhood population.

Acknowledgements

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References


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