The prevalence of antimicrobial resistance in human faecal flora in South Africa

P. M. A. SHANAHAN¹, B. A. WYLIE², P. V. ADRIAN², H. J. KOORNHOF², C. J. THOMSON¹ AND S. G. B. AMYES¹*

¹Department of Medical Microbiology, The Medical School, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, Scotland 
²Emergent Pathogen Research Unit of the Medical Research Council, University of the Witwatersrand and the South African Institute for Medical Research, Johannesburg 2000, South Africa

(Accepted 17 April 1993)

SUMMARY

Between January and March 1992, 361 faecal specimens were collected from the healthy black population in the Transvaal Province of South Africa. Each specimen was examined for the prevalence of antimicrobial resistance in commensal bacteria. Volunteers, from both rural and urban dwellings, were divided into four age groups. The overall carriage rate of resistance varied from 88.6% for ampicillin, 74.2% for trimethoprim, 52.6% for chloramphenicol, 10.2% for nalidixic acid to 7.5% for gentamicin. The carriage of resistance found to each individual antimicrobial agent was slightly higher in the rural population rather than the urban population but there was no correlation between the prevalence of antimicrobial resistance and the age group.

INTRODUCTION

The increasing prevalence of bacterial resistance to most antibiotics is of concern as the choice of agents available for treatment becomes limited and inevitably more complicated. Furthermore, the potential exists where no available therapeutic agent would be effective against many pathogens [1].

The incidence of antibiotic resistance amongst clinical isolates is well documented. Antibacterial drug resistance has been shown to be higher in the developing world rather than the developed world [2]. Trimethoprim resistance amongst pathogenic Gram-negative bacteria was found to be 64% in South India [3], 49.1% in South Africa [4] and 63.3% in Nigeria [5]. In contrast, the levels of such resistance were found to be only 14–19% in Finland [6] and 23% in Scotland [7]. Reasons for the high levels of resistance in the developing countries may include the availability of antibiotics without prescription and the contamination of the water supply [8].

In contrast, the incidence of such resistance amongst the normal non-pathogenic

* All correspondence and reprint requests should be sent to Professor S. G. B. Amyes, Department of Medical Microbiology, The Medical School, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, Scotland.
flora such as *Escherichia coli* of healthy individuals, in the absence of concurrent or recent antibiotic consumption, has been less extensively recorded [8–11]. The carriage of resistance determinants in these organisms may complicate treatment as the potential exists firstly, for their transfer to any invading pathogens and secondly, the resistant bacteria may cause an endogenous infection [12].

It is recognized that transfer of resistance factors is encouraged when antibiotics are administered [13]. The acquisition of resistant bacteria may be achieved through ingestion of contaminated food, person-to-person or animal-to-man transmission [14, 15]. A number of sources have been associated with the emergence of resistant bacteria including developing countries [12], clinical practice, animal feedlots [16] and, more recently, day-care centres (DCC) [17].

The normal commensal flora is increasingly recognized as a reservoir of antibiotic resistance. As in clinical isolates, levels of resistance in this group appear to be higher in the developing rather than developed countries. This study examines resistance in the commensal faecal flora in the black population of South Africa. This study area represents a unique situation in having third-world living conditions with two important exceptions: there is generally no unprescribed use of antibiotics and the water supply is often bacteria-free.

**METHODS**

**Population description**

The survey participants consisted of healthy volunteers resident in the Transvaal, South Africa. Candidates were excluded from the study if they had received any medical treatment in the 3 weeks prior to sampling. This time period allowed any changes in faecal flora, resulting from antimicrobial usage, to be reversed [9, 18]. A completed questionnaire accompanied each specimen; it supplied information regarding the donor’s age, sex, address, medical history and data on social conditions. The study examined eight separate population groups from both urban and rural areas. In the urban area the four groups comprised infants attending either a childminder or a crèche in SOWETO (0–5 years), urban children (6–11 years) and urban teenagers (12–19 years) attending a school in Kagiso, a town on the West Rand, and urban adults (> 19 years) who resided in SOWETO. The rural population was composed of infants attending a ‘well baby’ clinic at Middleplaas in the KaNgwane district, rural children and rural teenagers attending a school in Hekpoort in the Magaliesburg district and lastly adults resident in KaNgwane district. Notably, the rural adults were all associated with the hospital at Shongwe Mission and consequently were in contact with patients. This specific population consisted of healthy mothers feeding sick children, mothers attending a nutrition centre for children and finally escorts to patients visiting the out-patients’ department. Parental consent was obtained for all persons involved of < 18 years.

**Sample collection and processing**

The survey collection was performed between January and March 1992; a total of 361 specimens were obtained. Rectal swabs or freshly passed faecal specimens were deposited in small plastic containers for immediate transport to the...
Antimicrobial resistance in faecal flora

laboratory. Following the protocol established for such studies [8] each faecal specimen was plated onto Oxoid MacConkey Agar plates containing either ampicillin (10 mg/l), nalidixic acid (10 mg/l), chloramphenicol (10 mg/l), gentamicin (4 mg/l) or no antibiotic. Each specimen was also plated onto Modified Difco Mueller Hinton Agar plates containing trimethoprim (10 mg/l) [19]. The plates were incubated overnight at 37 °C. Each plate was scored for the presence or absence of bacterial colonies which were subsequently classified as either lactose fermenters or lactose non-fermenters.

Water sample collection and processing

From each study centre, except for Kagiso, a 100 ml sample of the normal drinking water was collected in a sterile bottle. After immediate transportation to the laboratory, the water was filtered and the filter pad placed on an Oxoid MacConkey agar plate containing no antibiotic. The plate was incubated overnight at 37 °C. Each plate was examined for the presence of bacterial colonies which were subsequently identified.

Bacterial identification

Bacterial colonies were identified by standard biochemical analysis. Each strain was allocated to one of four groups; E. coli, Klebsiella species, Enterobacter species, or other enterobacteria.

Conjugation studies

All the isolates resistant to ampicillin and trimethoprim were tested for the ability to transfer their resistance determinants by the method of Amyes and Gould (1984) [19]. The conjugation experiments employed the rifampicin-resistant E. coli K-12 strain J62-2 [20] as the recipient.

RESULTS

Study population

A total of 361 participants were included from both the rural (183) and urban (178) studies. The male/female distribution was 1:1-4 in both the groups. The adult population was predominantly female (Table 1).

Frequency of antibiotic resistance

The results of the survey are shown in Table 2. A high proportion of volunteers carried bacteria resistant to antibiotics; in particular to ampicillin (88-6%) and trimethoprim (74-2%). Five antibiotics were screened in the study and, for each, the corresponding level of resistance identified was slightly higher amongst the rural populations. There was no difference in the level of resistance to each antimicrobial agent and age group except that gentamicin resistance in the rural adults was much higher than it had been amongst their urban counterparts (38-6% versus 4%).

Identification of ampicillin- and trimethoprim-resistant strains

All the ampicillin and trimethoprim resistant strains were identified (Table 3). E. coli represented the largest proportion of resistant bacteria when either susceptibility to ampicillin or trimethoprim was measured.
Table 1. Volunteers in the study

<table>
<thead>
<tr>
<th>Location/study group</th>
<th>Total no. of individuals</th>
<th>No. of females</th>
<th>Range (years)</th>
<th>Mean (years)</th>
<th>SD</th>
<th>Mode meat/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants</td>
<td>50</td>
<td>29</td>
<td>0-5</td>
<td>0.75</td>
<td>0.82</td>
<td>3</td>
</tr>
<tr>
<td>Children</td>
<td>47</td>
<td>26</td>
<td>6-11</td>
<td>9.1</td>
<td>1.42</td>
<td>3</td>
</tr>
<tr>
<td>Teenagers</td>
<td>36</td>
<td>19</td>
<td>12-19</td>
<td>14.0</td>
<td>1.24</td>
<td>3</td>
</tr>
<tr>
<td>Adults</td>
<td>50</td>
<td>35</td>
<td>&gt;19</td>
<td>28.4</td>
<td>10.12</td>
<td>3</td>
</tr>
<tr>
<td>Urban study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants</td>
<td>45</td>
<td>23</td>
<td>0-5</td>
<td>2.4</td>
<td>1.28</td>
<td>4</td>
</tr>
<tr>
<td>Children</td>
<td>42</td>
<td>18</td>
<td>6-11</td>
<td>9.1</td>
<td>1.69</td>
<td>4</td>
</tr>
<tr>
<td>Teenagers</td>
<td>47</td>
<td>22</td>
<td>12-19</td>
<td>13.1</td>
<td>0.93</td>
<td>7</td>
</tr>
<tr>
<td>Adults</td>
<td>44</td>
<td>42</td>
<td>&gt;19</td>
<td>33.0</td>
<td>6.06</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Percentage of specimens with organisms resistant to five antibiotics in South Africa

<table>
<thead>
<tr>
<th>Antibiotic*</th>
<th>Urban</th>
<th>Rural</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Ap</td>
<td>Tp</td>
</tr>
<tr>
<td>0-5</td>
<td>94</td>
<td>80</td>
</tr>
<tr>
<td>6-11</td>
<td>89.4</td>
<td>72.3</td>
</tr>
<tr>
<td>12-19</td>
<td>75</td>
<td>61.1</td>
</tr>
<tr>
<td>&gt;19</td>
<td>84</td>
<td>68</td>
</tr>
<tr>
<td>Total</td>
<td>86.3</td>
<td>71</td>
</tr>
</tbody>
</table>

* Ap, ampicillin; Tp, trimethoprim; Cm, chloramphenicol; NA, nalidixic acid; Gm, gentamicin.

Table 3. The incidence of organisms isolated from the study

<table>
<thead>
<tr>
<th>Organism</th>
<th>Ap-resistant strains</th>
<th>Tp-resistant strains</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>445 (73)</td>
<td>297 (83)</td>
</tr>
<tr>
<td>Klebsiella species</td>
<td>104 (17)</td>
<td>46 (13)</td>
</tr>
<tr>
<td><em>Citrobacter/Enterobacter</em>species</td>
<td>35 (6)</td>
<td>8 (2)</td>
</tr>
<tr>
<td>Other</td>
<td>24 (4)</td>
<td>6 (2)</td>
</tr>
</tbody>
</table>

Frequency of transfer

A total of 608 ampicillin resistant strains and 357 trimethoprim resistant strains were collected from the survey. The results of the transfer experiments show that only 26% of the ampicillin-resistant isolates contained self-transmissible plasmids. Of the isolates containing these plasmids 80.6% were *E. coli*, 12.5% were *Klebsiella* species and 6.9% were other. In contrast, 51.2% of trimethoprim-resistant isolates contained self-transmissible plasmids while a further 34% contained trimethoprim-resistant plasmids which could be mobilized with X* factor. Trimethoprim-resistant strains possessing self-transmissible plasmids were identified as 88.1% *E. coli*, 89% *Klebsiella* species, 10% *Enterobacter* species and 20% others.
Table 4. Social conditions of volunteers in the survey

<table>
<thead>
<tr>
<th>Location</th>
<th>Mean no./family</th>
<th>Mean no./house</th>
<th>% With animals</th>
<th>% Water source tap</th>
<th>Water contaminated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hekpoort</td>
<td>7.0</td>
<td>6.2</td>
<td>61</td>
<td>79</td>
<td>+</td>
</tr>
<tr>
<td>Shongwe</td>
<td>7.4</td>
<td>7.7</td>
<td>41</td>
<td>88</td>
<td>+</td>
</tr>
<tr>
<td>Urban study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOWETO</td>
<td>8.6</td>
<td>8.7</td>
<td>68</td>
<td>99</td>
<td>-</td>
</tr>
<tr>
<td>Kagiso</td>
<td>6.0</td>
<td>6.3</td>
<td>50</td>
<td>100</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT, not tested.

Environmental conditions

In an attempt to establish if environmental conditions exerted any influence over the carriage of antibiotic resistance in bacteria, various factors were examined.

Water supply. A representative water sample was taken in each study area except in Kagiso (Table 4). Screening in the urban locations revealed the water, which is municipally piped, to be free of any bacteria. In contrast, the water tested from Middleplaas clinic, stored rain water, contained *Pseudomonas aeruginosa*, *Serratia marcescens* and a Gram-positive coccus. The school in Hekpoort is provided with water from a bore hole. This was contaminated with four different bacteria, *Bacillus cereus*, *Acinetobacter anitratus*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*. The rural adult population was not exposed to a contaminated water supply as the water utilized at Shongwe hospital was filtered and chlorinated river water. This water was completely bacteria-free.

Living conditions. The social conditions of the participants are reflected in Table 4. In both the rural and urban study, approximately 50% of the participants were associated with animals. Despite the possibility of animal-to-man transmission of bacteria, this was probably not a major contributory factor in the spread of resistance as resistant bacteria were as commonly observed in participants living in the absence of animals.

Population density. The most influential environmental factors observed were the number of people per family and per house. Many of the rural homes were wattle and daub huts with only one room for sleeping. Similarly, it was not uncommon for many urban homes to have just one bedroom. As similar numbers of residents per house (mean = 6.0 people/house) were recorded in both the rural and urban areas, it would seem that this widespread over-crowding is a large contributor to the high levels of resistance.

DISCUSSION

Commensal flora represent an increasingly well-recognized reservoir of resistance genes. In this study the carriage rate of antimicrobial resistance amongst the commensal flora of selected black South African populations was examined. The incidence of antibiotic resistance carriage was 88.6% for ampicillin and 74.2% for trimethoprim. Chloramphenicol resistance was found to be lower at 52.6%
while the carriage of gentamicin and nalidixic acid resistance were lower still at 7.5\% and 10.2\% respectively. This lower carriage of resistance may reflect the lower usage of these antimicrobials as opposed to ampicillin and trimethoprim which are the most widely used antibacterials in the study areas.

The highest incidence to date for the carriage of resistant bacteria within commensal populations was recently reported from South India [8]; trimethoprim- or ampicillin-resistant bacteria were present in 98\% of the specimens screened. The factors contributing to the maintenance of this high carriage of bacterial resistance included the contamination of the water supply and the inappropriate dispensation and widespread use of antimicrobial agents [8]. The causal relationship between increased antibiotic resistance and increased antibiotic usage has been explained before.

It has been reported that the carriage of antibiotic-resistant strains is much higher amongst children than adults [13]. Possible reasons for this include high antimicrobial prescribing amongst this group and lower standards of hygiene which facilitate the transmission of resistant organisms. Examination of the resistance levels within our population sub-groups, however, revealed a lack of correlation between age and antibiotic-resistant faecal flora. This compares with the commensal study of Dutch urban communities which reported the same finding [21]. However, this contrasts with other findings: examination of child day-care centres (DCC) [17] revealed substantially higher levels of trimethoprim-resistant faecal flora (30\%) amongst the children attending the centre than is prevalent in the general population (6\%). Similarly, ampicillin-resistant faecal flora was reported as 70\% in this particular population which again is substantially higher than corresponding levels identified in the healthy community (34.9\%) [9]. In the current study, approximately half the urban infants attended a child-minder in SOWETO, the equivalent of a child day-care centre, and yet the levels of resistance observed in this particular group are not noticeably higher than any other sub-group. This suggests the spread of antibiotic resistance factors may occur at the same frequency in all strata of society.

One of the distinctions between the developed and developing world is the lack of an uncontaminated potable water supply in the latter. This has obvious implications in facilitating the spread of infectious disease and had been speculated to have a role in the transmission of antibiotic-resistant organisms [8]. This study reports contamination of the water and unsanitary conditions especially in rural areas which create the opportunity for cross-infection between bacteria [5, 8, 22]. However, in contrast to the Indian study which indicated contamination of the water supply coupled with high antimicrobial usage as being responsible for the high resistance levels in that area, these factors may only be contributors to the resistance levels witnessed in South Africa. This is because in spite of very basic conditions in the urban areas of SOWETO, all our participants came from houses with a tap supplying an uncontaminated supply of water. Surprisingly, although the water was contaminated in rural areas, it did not seem to affect the carriage of antibiotic-resistant *E. coli*; *E. coli* were not isolated in the water samples. However, ablution facilities in the rural villages were extremely limited increasing the opportunity for spread of resistant determinants through the faecal/oral route.
Antimicrobial resistance in faecal flora

In addition, unprescribed use of antimicrobial agents may be a contributing factor to the high levels of resistance found in the developing world. Although the ability to buy antibiotics over the counter may contribute to high-level resistance [5, 8, 22], such purchasing is not usually possible in South Africa [4]. However, there might be a higher usage of antibiotics associated with this population group in order to treat endemic diseases.

It is probable that the main influence determining and maintaining the high level of resistance in the black South African population is the severe overcrowding in the homes (> 6-0 people/house). Close contact facilitates the transmission of resistant determinants [23]. Intrafamilial contact has been shown to be an important factor in the spread of resistant determinants [24].

Once established, poor living conditions and poor sanitation may be the major contributors in maintaining the high levels of resistant bacteria in this reservoir. Indeed, these factors may become more important than antibiotic usage itself.

ACKNOWLEDGEMENTS

We should like to thank Lilly Industries Ltd and the Society of General Microbiology for providing the funds for research in South Africa. We are very grateful to Mrs Z. Kumalo, Dr and Mrs A. R. P. Walker and Dr Mark Barry for organizing the collection of the samples in the different areas. We thank Dr Frank Wegerhof for allowing us to utilize his laboratory in Shongwe. We should like to thank the Faculty of Medicine, University of Edinburgh for the Davidson Scholarship for P. M. A. Shanahan.

REFERENCES


