Influence of population density on antibiotic resistance

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Antibiotic consumption and population density as a measure of crowding in the community were related to the prevalence of antibiotic resistance of three cities in three different countries: St Johns in Newfoundland (Canada), Athens in Greece and Groningen in The Netherlands. Antibiotic consumption was expressed in DDD (defined daily dose), as DID (DDD/1000 inhabitants/day) and as DSD (DDD/km²). The prevalence of antibiotic-resistant Escherichia coli and enterococci was determined in faecal samples of healthy volunteers. In both Newfoundland (28 DID) and Greece (29 DID) the overall consumption of antibiotics was more than three times higher compared with that of The Netherlands (9 DID). The lowest prevalence of resistant E. coli against the majority of antibiotics tested was found for the samples from Newfoundland and was significant (P < 0.05) for cefazolin, oxytetracycline and trimethoprim. A poor correlation between the number of DID and the prevalence of resistance was observed [the Pearson correlation coefficient (Pcc) ranged between –0.93 and 0.87]. However, when population density was taken into consideration and antibiotic consumption was expressed in DSD, a strong correlation was observed (and Pcc ranged between 0.86 and 1.00). This study suggests that population density is an important factor in the development of antibiotic resistance and warrants special attention as a factor in resistance epidemiology.

Keywords: population density, antibiotic resistance, antibiotic use, E. coli, enterococci

Introduction

Several studies have found a positive association between antibiotic use and antibiotic resistance in the community.1–4 However, this is not a direct relationship and therefore other factors must contribute to the degree of antibiotic resistance observed in a population.5,6

Besides selection pressure (antibiotic use), dissemination of resistant bacteria and resistance genes between individuals further increase the prevalence of antibiotic resistance in a population.7 Migration of people and increase in population density through urbanization leads to many opportunities for interpersonal contact, facilitating the spread of resistant bacteria and resistance genes,8 and may be an important factor in the dissemination of antibiotic resistance. It is well recognized that dissemination of antibiotic-resistant microorganisms from patient to patient directly, via instruments or via health care workers, is an important cause of hospital-acquired infections,9 but it also facilitates the transmission of resistant bacteria and genes. Analogous to the hospital situation where patients live closely together in a relatively small area, population density may be considered a crowding factor outside the hospital.

In the present study, antibiotic consumption and population density were related to the prevalence of antibiotic-resistant faecal Escherichia coli and enterococci of healthy volunteers living in three cities from three different countries, i.e. Canada, Greece and The Netherlands. E. coli and enterococci
cocci were used as indicator bacteria, because they represent species of the main facultatively aerobic intestinal flora in humans.10–12

Materials and methods

Demographic data

The population density of the cities St Johns (Newfoundland, Canada), Athens (Greece) and Groningen (The Netherlands) was measured using official published information from the respective governmental authorities. Antibiotic consumption for Newfoundland was obtained from IMS Health. Antibiotic consumption in Greece was derived from Pharmetrica S.A., a subsidiary of the National Drug Organization of Greece. The over-the-counter use of antibiotics in Greece was included in the amounts measured. Antibiotic use in The Netherlands was obtained from the Scientific Institute of Dutch Pharmacists (SFK). Antibiotic consumption in the community was calculated for the province of Newfoundland in 1998 and for the countries Greece and The Netherlands in 1999. The total consumption included the following classes of antibiotics: penicillins, cephalosporins, macrolides/lincosamides, tetracyclines, trimethoprim/sulphonamides and fluoroquinolones (Table 1).

Antibiotic use was expressed as the number of defined daily doses (DDD) consumed per 1000 inhabitants per day (DID) and as the number of DDD consumed per km² per day (DSD). The number of DID multiplied by the population density (the number of inhabitants per km²) is the number of DDD per km² per day.

Study population

Faecal samples from residents of the cities St Johns, Athens and Groningen were obtained using randomly selected addresses from the telephone directory. In each city 600 residents were addressed, expecting a response of ~30%. The countries and respective cities were chosen because of their variety in antibiotic use and population density. Requests with sample bottles were mailed to the addresses with a letter stating the purpose of the study.

Culture methods

The participants were asked to mail or bring their sample on the day of collection to a local laboratory where 2 g of each sample was diluted 1:10 in 0.9% (w/v) NaCl supplemented with 20% (v/v) glycerol. The participants in Groningen sent their samples directly to the microbiological laboratory of the University Hospital Maastricht.

The diluted samples were stored at −20°C in the local laboratories and subsequently those of St Johns and Athens were transported on dry ice to the microbiological laboratory of the University Hospital Maastricht.

All further microbiological processing was done in Maastricht, as described previously.13 In short, after thawing the samples were further diluted. For E. coli, 40 µL of 10⁻² and 10⁻⁴ dilutions in 0.9% saline were inoculated on Levine agar plates (EMB-eosin methylene blue agar, Oxoid CM69; Basingstoke, UK) with and without antibiotics using a spiral plater (Eddy Jet, IUL Instruments, I.K.S., Leerdam, The Netherlands). For enterococci, KP-Streptococcus agar plates (Oxoid CM701) were inoculated with 40 µL of 10⁻¹ and 10⁻³ dilutions with and without antibiotics. The antibiotic concentrations used in the agar plates for both E. coli and enterococci were based on NCCLS guidelines, and are presented in Tables 2 and 3 for E. coli and enterococci, respectively. E. coli grows on Levine agar as purple colonies with a black centre and metallic shine. Only these colonies were counted after 18–24 h incubation at 37°C. For identification, a colony was randomly picked from the agar plate and was tested for indole and β-glucuronidase reaction. Previous studies have shown that E. coli isolates had identical or higher MICs than the antibiotic concentration of the selective plate they were isolated

Table 1. Antibiotic use in DID and DSD for the province Newfoundland and the countries Greece and The Netherlands

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Newfoundland</th>
<th></th>
<th>Greece</th>
<th></th>
<th>The Netherlands</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DID</td>
<td>DSD</td>
<td>DID</td>
<td>DSD</td>
<td>DID</td>
<td>DSD</td>
</tr>
<tr>
<td>Aminopenicillins</td>
<td>11</td>
<td>3</td>
<td>6</td>
<td>52</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Other penicillins</td>
<td>3</td>
<td>0.7</td>
<td>4</td>
<td>33</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>49</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Macrolides and lincosamides</td>
<td>4</td>
<td>0.9</td>
<td>7</td>
<td>57</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>3</td>
<td>0.8</td>
<td>0.7</td>
<td>5</td>
<td>0.8</td>
<td>2</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>1</td>
<td>0.4</td>
<td>3</td>
<td>21</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>2</td>
<td>0.5</td>
<td>2</td>
<td>12</td>
<td>0.8</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>7</td>
<td>29</td>
<td>229</td>
<td>9</td>
<td>20</td>
</tr>
</tbody>
</table>
Enterococci appear as typical red or pink colonies on KF-Streptococcus agar. After 48 h incubation at 42°C only the typical pink colonies were counted. The randomly picked enterococci colonies were identified by using tolerance to bile, esculin hydrolysis, growth in 6.5% (w/v) NaCl and a positive pyrrolidonylarylamidase reaction (Wellcome). A previous study has shown that enterococci isolates had MICs that were identical to or higher than the antibiotic concentration of the agar plate they were selected from. The prevalence (%) of antibiotic resistance was defined as the number of faecal samples showing growth of resistant E. coli or enterococci divided by the total number of faecal samples tested multiplied by 100. The degree of resistance of each sample was defined as the ratio (%) between the number of colonies grown on the agar plates with and without antibiotics.

The study was approved by the Medical Ethics Committee of the University Hospital Maastricht (no. 97-1-104).

### Statistical analysis

The $\chi^2$ test was used to determine the significant differences ($P \leq 0.05$, two-sided) in the prevalence of antibiotic resistance between the three different populations. For a valid $\chi^2$ test, all expected frequencies had to be $\geq 1$ and no more than 20% of the expected frequencies may be $<5$. If the data did not fulfil these criteria, the significant differences were invalid and therefore not displayed.

Pearson’s coefficient of correlation (Pcc) was used to assess the correlation between antibiotic consumption—expressed by the number of DID and DSD—and antibiotic resistance between the three populations. All analyses were carried out in SPSS for Windows.

### Results

#### Demographic data

The population densities of the cities St Johns, Athens and Groningen were 255 (127 482 inhabitants in 499 km$^2$), 8194 (3523407 inhabitants in 3351 km$^2$) and 2046 (171193 inhabitants in 84 km$^2$) inhabitants/km$^2$, respectively. The population density of Athens was four times higher than Groningen and 32 times higher than St Johns.
Study population

Faecal samples were obtained from 154, 179 and 129 residents from St Johns, Athens and Groningen, with a response rate of 26%, 30% and 29%, respectively. The mean age (S.D.) of the participants was 55 (15), 47 (21) and 55 (18), respectively.

Antibiotic use

As shown in Table 1, the total use of antibiotics in DID was three times higher for Newfoundland (28 DID) and Greece (29 DID) compared with The Netherlands (9 DID), whereas the total observed use in DSD was the lowest for St Johns (7 DSD) followed by Groningen (20 DSD) and by far the highest for Athens (229 DSD).

In Newfoundland, more aminopenicillins (11 DID), trimethoprim (3 DID) and fluoroquinolones (2 DID) were used than in the other countries, whereas Greece displayed the highest use for ‘other’ penicillins (4 DID), cephalosporins (6 DID), macrolides (7 DID) and tetracyclines (3 DID). The Netherlands showed the lowest consumption for most antibiotic groups, except for trimethoprim and tetracyclines (Table 1).

For all antibiotic groups, Athens clearly showed the highest numbers of DSD, and St Johns the lowest, except for cephalosporins, which was lowest in Groningen (0.2 DSD) (Table 1).

Prevalence of antibiotic resistance

E. coli were detected in 110 (71%), 148 (83%) and 112 (87%) of the faecal samples, respectively, and enterococci were isolated from 116 (75%), 177 (99%) and 112 (87%) of the faecal samples for St Johns, Athens and Groningen, respectively. In general, the prevalence of resistance for E. coli and enterococci for almost all agents tested was highest in Athens, followed by Groningen, and was lowest in St Johns (Tables 2 and 3).

E. coli. The prevalence of amoxicillin, chloramphenicol and trimethoprim resistance was significantly higher ($P \leq 0.05$) in Athens compared with St Johns. The resistance prevalence for cefazolin, nalidixic acid and oxytetracycline was significantly higher ($P \leq 0.05$) in Athens compared with both St Johns and Groningen. Resistance to cefazolin, trimethoprim and oxytetracycline was significantly higher in Groningen compared with St Johns. No nitrofurantoin resistance was observed in the three cities (Table 2).

Enterococci. The prevalence of resistance to ciprofloxacin, erythromycin and oxytetracycline was significantly higher ($P \leq 0.05$) in Athens than in St Johns and Groningen. In Groningen, resistance to erythromycin and oxytetracycline was significantly higher than in St Johns (Table 3).

Relationship between antibiotic resistance and antibiotic use

For the majority of antimicrobial agent and resistance combinations in the present study, a better correlation was found when antibiotic consumption was expressed in DSD compared with DID (Table 4). For example, the prevalence of amoxicillin-resistant E. coli correlated much better with the number of aminopenicillins in DSD (Pcc = 0.89) than when expressed in DID (Pcc = –0.39) (Table 4, Figures 1 and 2).

Discussion

The participation rate (26–30%) and the mean age of participants were in the same order in all three cities (55, 47 and 55 years). The relatively low response can be partly explained by the embarrassment of sending in a faecal sample.

<table>
<thead>
<tr>
<th>Table 4. The Pearson correlation between the prevalence of resistance and antibiotic use in DID and DSD for the different regions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antimicrobial agent</strong></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td><strong>E. coli resistance</strong></td>
</tr>
<tr>
<td>amoxicillin</td>
</tr>
<tr>
<td>trimethoprim</td>
</tr>
<tr>
<td>nalidixic acid</td>
</tr>
<tr>
<td>cefazolin</td>
</tr>
<tr>
<td>oxytetracycline</td>
</tr>
<tr>
<td><strong>Enterococci resistance</strong></td>
</tr>
<tr>
<td>erythromycin</td>
</tr>
<tr>
<td>oxytetracycline</td>
</tr>
</tbody>
</table>
Newfoundland and Greece, the overall consumption of antibiotics (DID) was three times higher compared with that of The Netherlands. However, the lowest prevalence of resistance for the majority of antibiotics tested was found for the samples from Newfoundland. This was significant for cefazolin-, oxytetracycline- and trimethoprim-resistant *E. coli*, and erythromycin- and oxytetracycline-resistant enterococci.

Antibiotic use is generally considered as the major factor determining the prevalence of antibiotic resistance in a population. Another important factor contributing to the development of antibiotic resistance is the dissemination of resistant bacteria or resistance genes from a resistant donor to a susceptible host, which subsequently can either become colonized by these bacteria and/or these bacteria might transfer their resistance genes to bacteria belonging to the intestinal flora of the new host during their passage through the intestinal tract. In the hospital, it is generally accepted that patients either colonized or infected with multiresistant microorganisms must be isolated to prevent spread of the resistant microorganisms to other patients. Outside the hospital, a higher prevalence of resistance was observed among persons living in close contact with pigs and poultry, which have a very high prevalence and degree of resistance in their intestinal flora due to the excessive use of antimicrobials as growth promoters and therapy in animal husbandry, i.e. farmers, compared with urban residents living in the same geographic region.18

This clearly shows that living in an environment with resistant bacteria is a real risk for acquiring such bacteria and that the closer the contact the larger the risk. Similar to the hospital environment and living in close contact with food animals, one could speculate that in the community living in close contact with other persons harbouring resistant microorganisms or resistance genes is a risk factor for the acquisition of antibiotic resistance. The commonly observed high levels of resistance among intestinal colonizers and clinical isolates in the developing world support cross-infection not only from crowding but mainly from poor sanitation as an important means of sharing resistant bacteria in many parts of the world.12,19 However, it seems unlikely that in this study differences in hygiene standards have significantly influenced the prevalence of resistance, since Canada, The Netherlands and Greece are all developed countries with high and similar sanitation standards.

The results of this study indicate that next to antibiotic consumption population density is a factor influencing the prevalence of antibiotic resistance in the faecal flora of healthy individuals in populations. Using population density as a measure of crowding in the community, we found a good correlation between the number of DSD and the prevalence of resistance for the majority of antibiotics. This suggests that the chance of a susceptible host picking up resistant bacteria or resistance genes from a resistant neighbour is higher in areas with a high population density (like Groningen and Athens), than in areas with a low population density like St Johns. Resistant organisms can only spread if (susceptible) hosts are in the neighbourhood. In an area with high antibiotic use by several persons living far apart from each other (St Johns), the contribution of the transfer/spread of resistant bacteria to a susceptible host is lower than in an area where people live next/close to each other.

Not all drug resistance combinations gave a better correlation when the prevalence of resistance was related to DSD instead of DID. Comparable Pcc values were observed for oxytetracycline and macrolides. This could be explained by the existing high prevalence of resistance to these agents both in Athens and Groningen. Also the ability of resistance mechanisms to spread can differ, and more mobile genes might need lower population density to disseminate. In the present study, however, no molecular analyses were carried out to detect the actual resistance mechanisms of the isolated organisms.

In our attempt to indicate the importance of crowding in the community as a factor to facilitate dissemination of resistant bacteria and resistance genes to susceptible hosts, we recognize that this study has several limitations. Only three places were involved and surely other factors play a role in the development of resistance besides antibiotic use and population density. For instance, the warmer climate in Athens compared with St Johns and Groningen promotes the growth
of (resistant) enteric bacteria in the environment. Apart from human antibiotic consumption the differences in the amount and kind of antibiotics used in animal husbandry or for crop protection might also have influenced the prevalence of resistance in the three populations. Moreover, the correlation between antibiotic resistance and the use of a specific antibiotic agent or group could have been biased by multiple- or cross-resistance, as the use of one agent can maintain high levels of resistance to another. However, as a result of the magnitude of the differences in antibiotic use, population density and antibiotic resistance between the three places, we do not expect that, for example, the relatively low response rate, or the fact that antibiotic use was measured on a country/provincial basis and not regional, could have substantially influenced the study outcome.

In conclusion, this study implies that population density as a measure of crowding might be an important factor in the prevalence of antibiotic resistance in populations and warrants special attention in resistance epidemiology.

Acknowledgements

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References