Carriage of Antibiotic-Resistant Fecal Bacteria in Nepal Reflects Proximity to Kathmandu

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Within Nepal, geographic, social, and economic barriers greatly limit access to allopathic health care. The country therefore offered the opportunity to evaluate the effect of antibiotic accessibility (as measured by allopathic medicine consumption) on antibiotic resistance in the normal intestinal flora. The aerobic gram-negative fecal flora of 33–34 healthy adults from each of 3 villages with different access to health care facilities in Kathmandu were examined for antibiotic susceptibility. The frequency of antibiotic resistance decreased significantly with increasing distance from Kathmandu and decreasing population density but did not reflect contact with health care providers or individual medicine consumption. The findings suggest that an individual’s overall exposure to antibiotics and antibiotic-resistant bacteria (resulting from close proximity to other community members and to sources of accessible allopathic health care, such as in the vicinity of Kathmandu), has an equal or greater impact on an individual’s carriage of antibiotic-resistant bacteria than does direct consumption of antibiotics.

Antibiotic resistance has emerged as a significant threat to public health worldwide [1]. Although studies have documented the correlation between the consumption of antibiotics and the frequency of indigenous resistant organisms in hospitalized individuals [2–4] and in countries [5–7], similar studies in communities lacking easy access to allopathic health care are minimal [8, 9]. Reports have asserted that major factors driving the selection pressures responsible for the increasing frequency of antibiotic resistance are volume and length of time of drug use [6, 10]. However, other studies have demonstrated that resistant organisms can be present in individuals and in animals not taking antibiotics [3, 7, 9, 11–15]. One explanation attributes resistance selection to the density of individuals being treated in a particular geographic setting [16].

This concept takes into consideration not only direct selection by antibiotics but also the person-to-person spread of resistant strains. For instance, antibiotic resistance appeared among individuals who were not taking antibiotics but were sharing the same home [17] or day care center [18–19] with those consuming antibiotics. In one such study, there was a significant correlation between the use of antibiotics in various communities and the frequency of penicillin-resistant pneumococci in children in those same communities [20]. Another study showed that individuals sharing a household with those taking antibiotics for acne harbored significantly higher numbers of drug-resistant staphylococci on the skin than did a control group of individuals in homes of nontreated individuals [17].

Populations that are isolated from the influences of Western drugs and medicine are difficult to access and to study, yet they may provide important information to help us understand the origins, evolution, and spread of antibiotic-resistance genes. Such an opportunity was offered by a study of the resident fecal flora of 3 different Nepalese villages, each exhibiting different population densities and levels of accessibility to allopathic health care because of their geographic distance and relative isolation from the capital city of Kathmandu or other major urban centers.

Materials and Methods

Demographics and target population. Three villages at various distances from Kathmandu were selected for study (table 1). Dhulikhel, which is accessible from Kathmandu via a 30-min bus trip, provides the 11,000 inhabitants access to numerous hospitals, pharmacies, clinics, and allopathic physicians. Dolakha is located 6 h from Kathmandu on the same bus route as Dhulikhel. There are 2 hospitals and 6 pharmacies available to the 3500 villagers in Dolakha, as well as bus access to Kathmandu and the health ser-
Table 1. Demographics of Nepalese study populations in 3 Nepalese villages.

<table>
<thead>
<tr>
<th>Village</th>
<th>Approximate population</th>
<th>Distance from Kathmandu, km</th>
<th>No. of hospitals*</th>
<th>No. of health posts*</th>
<th>No. of pharmacies*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dhulikhel</td>
<td>11,000</td>
<td>31</td>
<td>6*</td>
<td>&gt;10</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Dolakha</td>
<td>3500</td>
<td>133</td>
<td>2*</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Simigaun</td>
<td>330</td>
<td>170</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Any facility within 1 h (walking or bus).

** Statistical analysis. ** Data were entered and statistics were performed using the SPSS software program (SPSS) and Epi Info software package (version 6.0, Center for Disease Control and Prevention). Frequency of resistance in gram-negative aerobic fecal flora for each of 8 different antibiotics was analyzed by use of analysis of variance, with a Student-Neuman-Keuls test, where applicable. Two levels of resistance (≥10% and ≥50% of the aerobic fecal flora) were analyzed. Multiple resistance profiles were determined on the replica-plated samples, using χ² analysis. Potential confounding, such as the use of medicine and visits to health care providers, was evaluated with independent sample Student’s t tests.

Results

Of the 50 swabs distributed in each village, 33–34 per village were returned to the investigators. This rate of sample return (66%–86%) may reflect that there was <24 h between distribution and collection. Not all volunteers may have produced a sample in that time period. In addition, some lack of return may be attributable to Nepalese cultural embarrassment concerning bowel habits. There was no difference in the time of return of the samples among the villages (15 ± 3 h).

The total population surveyed consisted of 38% males and 62% females, ranging in age from 15 to 47 years (mean, 25.5 years; table 2). The sex distribution among the participants was similar in Dolakha and Dhulikhel. The low percentage of males in Simigaun (9.1%) was presumably due to employment outside the village in the trekking industry or participation in regional political elections. There was no significant variation in the mean ages among villages.

About 61% of the samples showed only Lac⁺ (lactose fermenters) phenotypes on MacConkey agar. Thirty-nine percent of samples contained both Lac⁺ and nonfermenters (Lac⁻), but the latter constituted <15% of any given sample. Since the Lac⁻ population comprised a minor component of the total, this analysis focused on the profiles of the predominant Lac⁺ population. The recovery of *Salmonella* or *Shigella* species from HE was infrequent and occurred in ~1% of the samples recovered from the participants. Because of the low frequency, these isolates were not characterized further.
The frequencies of resistance to each drug in Lac⁺ colonies were compared among the samples from each of the different village populations. A comparison of results from males and females in Dolakha and Dhulikhel villages showed no significant difference between the sexes (data not shown). Consequently, the samples from males and females were combined. All samples showed some level of resistance to all the drugs tested, with the exception of Gm resistance, which was not found in Simigaun. The number of individuals with a ≥10% frequency level of resistance (≥10% of growth, compared with the master plate) to ≥1 antibiotic was similar for all 3 villages: 31 for Dolakha and Simigaun and 32 for Dhulikhel. However, significant differences in resistance to specific drugs between villages were found for Tet, CMZ, Cthn, NA, and Gm (figure 1A). The most profound of these was a 2-fold higher resistance to Tet and a 5-fold higher resistance to NA in the closest village (Dhulikhel), compared with results from either of the further villages (Dolakha and Simigaun; P < .05). High-frequency resistance (i.e., resistance in ≥50% of the organisms in an individual’s Lac⁺ flora) was significantly less prevalent in the distant villages than in Dhulikhel (figure 1B). Although 29 individuals (88%) from Dhulikhel demonstrated this high-frequency resistance to ≥1 antibiotic, only 16 individuals from Dolakha (47%, P = .001) and 22 from Simigaun (67%) demonstrated such high-frequency resistance. Samples from Dhulikhel (the closest village to Kathmandu) had a 2–3-fold higher frequency of bacteria resistant to Tet, CMZ, Cthn, and NA, compared with samples from the further villages (figure 1B).

Coreistance to ≥2 of the drugs within the same bacterial colonies of an individual’s flora (at a level of ≥10% of each sample) appeared in >90% of the samples from Dhulikhel and in <80% of samples from the other 2 villages (figure 2). Larger differences between the villages appeared with coreistance to ≥3 drugs (Dhulikhel, ≥90%; other villages, <60%). Resistance to ≥4 antibiotics was high in Dhulikhel (~70% of the sampled population), whereas it was markedly less in the other villages (<40%; figure 2). In fact, individuals from Dhulikhel had fecal organisms resistant to significantly more antibiotics, with a mean of 4.5 drugs (at ≥10% of each sample), compared with a mean of 3.1 drugs for Dolakha and 2.5 drugs for Simigaun (P ≤ .001; figure 2). At the ≥50% level, individuals from Dhulikhel bore resistance to a mean of 3.1 antibiotics, compared with 1.2 antibiotics for Dolakha and 1.0 antibiotic for Simigaun (data not shown). These differences were also highly significant (P ≤ .001).

Compared with reports from Dhulikhel and Dolakha, significantly fewer individuals in Simigaun had reported visiting an allopathic doctor within the previous year (P = .009) or within their lifetime (P ≤ .001; table 2), and no Simigaun villagers reported visiting an allopathic physician within the past month. Among the subset of villagers who had ≥50% resistance (as described above) to ≥1 antibiotic, these differences persisted: 89% of inhabitants from 2 villages closest to Kathmandu had visited a doctor within their lifetime, compared with only 50% of those from Simigaun (data not shown). This difference remained statistically significant (P ≤ .001).

A history of the past and present use of allopathic medications showed that, even in the most isolated village, there was exposure to these drugs, and most (>75%) individuals from each of the 3 villages had used them at some point in their lifetime. Neither the lifetime usage nor recent (past year) usage differed significantly among the villages (P = .059 and .098, respectively; table 2). In addition, a comparison of the subset composed of only those individuals who possessed highly resistant flora (i.e., had flora with ≥50% resistance to any drug) showed variable levels of contact with medications. Although 93% of these individuals from the 2 villages closest to Kathmandu reported use of medicines, only 77% of such individuals from Simigaun reported having used them (P ≤ .001; data not shown).

### Discussion

Antibiotic resistance is prevalent even among populations of people or animals who have had no recent antibiotic consumption [3, 7, 9, 11–15, 23–26]. Why this phenomenon exists is not clear. Some studies suggest that it relates to an environ-
Figure 1. Percentage of fecal samples from each of 3 Nepalese villages bearing drug-resistant, gram-negative, lactose-fermenting fecal organisms. A, Resistance frequency at the 10% level (defined as >10% of visible colonies able to grow when exposed to the antibiotics listed, using a replicating method). Statistically significant $P$ values ($P < .05$) existed for values between the 2 distant villages of Simigaun and Dolakha and the most urban village of Dhulikhel (*); the differences between the 2 distant villages and the furthest village, Simigaun (**). B, Resistance frequency at the 50% level (defined as >50% of visible colonies able to grow when exposed to antibiotics as listed, using a replicating method). Statistically significant $P$ values ($P < .055$) existed for values between the 2 distant villages of Simigaun and Dolakha and the most urban village of Dhulikhel (*); the differences between the 2 distant villages and the most urban village approached statistical significance ($P = .05$). Amox, amoxicillin; Chl, chloramphenicol; CMZ, cotrimoxazole; Cthn, cephalothin; Gm, gentamicin; NA, nalidixic acid; Nfx, norfloxacin; Tet, tetracycline.

Nepal, situated between India and Chinese Tibet, supports a population of >20 million people who are divided into >30 culturally diverse ethnic groups [28]. With 81% of the population occupied in farming, most Nepalese are primarily engaged in subsistence agriculture for their livelihood. The diverse geographical terrain of Nepal serves to demarcate the inhabitants into clearly defined urban (9%) and relatively isolated rural populations (91%), many of which are accessible only by footpath. Up to 85% of Nepalese, particularly in rural areas, rely primarily on traditional healing methods, such as those employed by Jhankri (traditional healers), who may use herbs but traditionally do not dispense antibiotics [29, 30]. Of all Nepalese who seek any type of medical care, ~10% consult first with a jhankri. This number reaches >20% in the Eastern Hill Region of Nepal [31]. The vast majority of allopathic health care services are centralized in major urban areas, such as Kathmandu, Pokhara, and Birgunj. For allopathic care, the inhabitants of rural areas often rely on health posts, which are minimally staffed and supplied.

Antibiotic use in Nepal is difficult to assess because of the lack of data on specific use. One study conducted in 1998 showed that 68% of drug prescriptions/recommendations for diarrhea and 70% of prescriptions/recommendations for acute respiratory infection symptoms were for antimicrobials. Of all encounters at health facilities and with private-sector pharmacists, 50.7% resulted in antimicrobials being dispensed [32]. With a physician-to-patient ratio of 1:16,667 (compared with a ratio of 1:420 in the United States), most Nepalese are forced to rely on health assistants and pharmacy shopkeepers as their
neither doctor visits nor consumption of medications differed of health care facilities (figure 1 and figure 2). Here, however, what similar population size but very different in the number resistance between Dhulikhel and Dolakha, which are of some-

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gaan (figure 1). The finding of higher levels of resistance to

to the fluoroquinolones) are more difficult to obtain. Resistance to all of these newer antibiotics (Gm, NA, and Nfx) was almost completely absent (<5% frequency) in the furthest village of Simigaun (figure 1). The finding of higher levels of resistance to newer agents in the more urban populations suggests that antib-
iotics like the fluoroquinolones are available in these areas.

Of particular note is the dramatic difference in frequency of resistance between Dhulikhel and Dolakha, which are of some-
what similar population size but very different in the number of health care facilities (figure 1 and figure 2). Here, however, neither doctor visits nor consumption of medications differed significantly. In fact, the village with less resistance (Dolakha) showed more recent doctor visits (65% vs. 42%) and more med-
ication use (68% vs. 48%; table 2).

The levels of resistance found in the fecal samples from the furthermorest village (Simigaun) were particularly interesting. The relative isolation of this village from hospitals and health posts makes contact with antibiotics through health care professionals relatively unlikely. In fact, none of the individuals from this remote village reported recent contact (within 1 month) with an allopatic physician, and most (52%) had never had such contact. Despite the lack of direct contact with health care workers, however, 87% of all villagers reported using al-
lopatic medicines at some point in their lifetime, and 53% reported having used them within the past year, although the exact identifications could not be defined. This information suggests that drugs were obtained from other sources, such as trekkers, traveling contacts, or drug-distribution programs.

In addition, a relatively large number of men from Simigaun were involved in the trekking industry. These men are frequently absent from the village for extended periods and have contact with outside tourists and inhabitants of urban centers, such as Kathmandu. Our survey purposefully excluded these individ-
uals as possible direct contributors. However, transfer of resis-
tant bacteria occurs both in the presence and the absence of antibiotic-selective pressure [11, 27]. Thus, trekkers could po-
tentially serve as transmission vectors to contacts in their home communities. In addition, more than half the 42 plant species recorded as widely available and commonly used in Nepalese herbal medicine are reported as having “antiseptic” or “dis-
fectant” qualities and may, therefore, contribute to the selec-
tion and propagation of drug-resistant bacteria [31]. Last, rural village dwellers frequently share a common water source for multiple purposes (i.e., drinking, bathing, laundry, and crop irrigation), and this may enhance the probability of shared bact-
erial strains among these populations. Unlike Dolakha and Dhulikhel, which have numerous water sources, Simigaun maintains a single common tap system, which is known to have many breaks with potential exposure to runoff.

Multidrug resistance (resistance to ≥2 antibiotics) was sig-
nificantly higher in Dhulikhel than in either of the more rural communities (figure 2). Although the low number of doctor visits could possibly explain the lower multiresistance found in Simigaun, it does not account for the similarly low multidrug resistance found in Dolakha (table 2). In addition, and more important, there was no significant difference in consumption of medicines that can explain the different multiresistance fre-
cuencies found in the 3 villages (table 2). There was not even a clear relationship between medicine use and resistance when comparing the subsets of all individuals who exhibited high-
level resistance (i.e., ≥50% of flora resistant to ≥1 drug). Al-
though 93% of those in Dolakha and Dhulikhel reported taking medication, only 77% of those in Simigaun reported doing so.

The finding of significantly higher proportions of multidrug

Figure 2. Total no. of antibiotics (0–8) to which individual samples
(gram-negative, lactose-fermenting fecal flora) from each village were resistant at the 10% level (defined as ≥10% of visible colonies able to grow when exposed to antibiotics, as listed in the figure 1 legend, using a replica-plating method). The comparison shows significant differences in the mean nos. of antibiotics to which organisms were resistant: Dhulikhel (mean, 4.5 antibiotics) and the other 2 villages (mean, <3.1 antibiotics; P < .001).
resistance in the population(s) closest to clusters of health care centers and hospitals parallels our previous report of higher multidrug resistance frequencies in a hospitalized population with intense exposure to antibiotics [3]. It likewise lends support to the suggestion that resistance correlates with the environmental density of antibiotic emanating from focal points of drug distribution.

These findings support the hypothesis that it is not only medicine consumption or doctor visits but rather the environment in which the villagers live that dictates the level of antibiotic resistance. It is striking (see figures 1 and 2) that Simigaun and Dolakha resemble each other in resistance frequency, compared with Dholikhel, despite the fact that they are of different sizes and at considerably different distances from the capital city. For the 11,000 inhabitants of Dholikhel, there are >60 health care facilities (≥1/166 persons), whereas Dolakha has only 9 for its 3500 villagers (1/389 persons), and Simigaun has none for 330 dwellers. Together, these data again support the hypothesis that it is the environmental density of antibiotics and antibiotic-resistant bacteria arising from foci of antibiotic availability that affects the net resistance frequency in the community population.

The higher frequencies of resistance to both single and multiple antibiotics in the fecal flora of villagers living closer to Kathmandu suggest that antibiotic availability was a key factor in determining the carriage of resistant fecal bacteria by an individual. However, the finding that this relationship between distance from Kathmandu and resistance frequency was independent of the individual’s report of doctor visits and allopathic medicine usage suggests that the close community environment of antibiotic use and potential for spread of resistant bacteria contribute as much or more to the frequency of resistant bacteria in an individual’s fecal flora than does the individual’s own antibiotic usage.

References

