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Resistance in faecal *Escherichia coli* isolated from pigfarmers and abattoir workers

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SUMMARY

Faecal samples collected from three populations of healthy adult volunteers (290 pigfarmers, 316 abattoir workers, 160 (sub)urban residents) living in the south of The Netherlands were analysed for the prevalence and degree of antibiotic resistance of *Escherichia coli*.

Significant differences in prevalence of resistance to amoxicillin, neomycin, oxytetracycline, sulfamethoxazole and trimethoprim were observed. The pigfarmers showed the highest percentages of resistance and the (sub)urban residents the lowest. In contrast no significant differences in high degrees of resistance were observed, except for neomycin.

Although both pigfarmers and abattoir workers have regular contact with pigs differences in prevalences of resistance were observed. However, because abattoir workers with intensive and less intensive pig(carcass) contact did not show significant differences, this is probably not the only important source of resistant *E. coli* in pigfarmers.

The high antibiotic use by pigfarmers (5%) and abattoir workers (8%) than by (sub)urban residents (0%) did not result in significantly different resistance percentages.

INTRODUCTION

Since the introduction of antimicrobial agents they have been successfully used to prevent and treat bacterial diseases in man and other species. The availability of antibiotics means that many previously severe infections can now be treated. In addition, antibiotics are used for growth promotion in animal husbandry and in agriculture for crop protection.

As antibiotics are not only very effective, but also remarkably safe drugs this safety may have provoked liberal, even lavish, use in man and other animals. The use of antibiotics, however, leads inevitably to emergence of resistance in the endogenous bacterial flora of treated persons and animals alike, against the antibiotics used or to other drugs [1, 2]. These enteric microorganisms may colonize other persons or animals and transfer resistance plasmids to their faecal flora. Consequently the environmental bacterial population may be contaminated after faeces excretion. Lester and colleagues [3] showed that persons with a few resistant bacteria in their intestinal flora will have more chance of developing an infection with resistant bacteria after antibiotic therapy than persons with no resistant strains at all.

Many studies have examined the resistance of enteric bacteria in humans after antibiotic therapy [4–8], but there is much less information available on the prevalence of antibiotic resistance in the faecal flora of healthy adults who have not used antibiotics recently [9–13]. However, such subjects are potential recipients of antimicrobial agents.

Farmworkers can directly become colonized by resistant bacteria due to close contact with animals and their faeces [14–16], but are also directly exposed to antibiotics used for treatment or prevention of diseases in animals [17]. Abattoir workers have daily contact with contaminated carcasses and gut contents [18–21]. A common risk factor for colonization with resistant microorganisms in all three groups is personal use of antibiotics.

To elucidate the importance of spread of resistance from food-animals to man we studied in the same region the antibiotic resistance in three populations with different risks of exposure to faecal bacteria from pigs, i.e. pigfarmers, abattoir workers and as a control group, (sub)urban residents.

As the faecal flora is considered the most important reservoir of resistant microorganisms and the antibiotic resistance of this flora is an indicator for the resistance of potentially pathogenic bacteria in a population [3, 4, 12, 22], faecal samples of these three populations were analysed for the prevalence and degree of antibiotic resistance of *Escherichia coli*.

MATERIALS AND METHODS

Collection of the faecal samples

Faecal samples, one from each person, were received from adult pigfarmers (290), pig-abattoir workers (316, of which 73 were meat inspectors) and (sub)urban residents (160), all living in the same area.

After receipt, the samples were diluted (10^{-1}) in physiological saline, containing 20% (v/v) glycerol and stored at −20 °C until examined. All participants were asked to answer a questionnaire concerning antibiotic use in the previous 3 months. Additional information about recent hospital stay and previous antibiotic use by family members was obtained from the pigfarmers and abattoir workers. The abattoir workers were also asked to give some information about keeping domestic animals or pigs and their daily duties at the slaughterhouse.

Bacteriological analysis of the faecal samples

The methods used to determine the prevalence and degree of resistance were as described before [23].

In brief, after thawing the samples (10^{-1}), tenfold dilutions (10^{-2}–10^{-5}) in physiological saline were made. Thirty-seven μl of these dilutions were inoculated with a spiral plater on Levine-agar plates (BBL 11221, [24]), a selective medium for *E. coli*, with and without antibiotics. The antibiotic concentrations (Table 1) were based on NCCLS guidelines and modified where appropriate so that the data were comparable with those of previous studies [9, 10]. Only colonies with the appearance of *E. coli* (i.e. purple with a black centre and a metallic green shine) were counted. The total number and the number of resistant *E. coli* were

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Table 1. *Prevalence and high degree of antibiotic resistant* Escherichia coli (%)

Antibiotics mg/l	Prevalence PF $n = 278$	Prevalence AW $n = 289$	Prevalence UR $n = 150$	High degree PF $n = 278$	High degree AW $n = 289$	High degree UR $n = 150$
AMX (25)	62	42*	47§**	7	9	13
AP (32)[a]	3	1	nt	0	0	nt
CIP (4)	1	0	0	0	0	0
NA (32)	5	3	1	1	0	0
NE (8)	66	36*	25§**	7	2*	2§**
FT (50)	8	4	3	0	0	0
OT (25)	79	47*	36§‖**	10	15	8
SMX (100)	84	45*	40§**	17	13	10
TMP (8)	53	23*	15§**	4	4	3

AMX, amoxicillin; AP, apramycin; CIP, ciprofloxacin; NA, nalidixic acid; NE, neomycin; FT, nitrofurantoin; OT, oxytetracycline; SMX, sulfamethoxazole; TMP, trimethoprim.

PF, pigfarmers; AW, abattoir workers; UR, (sub)urban residents; nt, not tested.

[a] Apramycin was only tested for the slaughterhouse workers and the last 116 farmers faecal samples.

Significantly different ($P \leqslant 0.05$); * PF and AW; § PF and UR; ‖ AW and UR; ** PF and AW and UR.

determined. The minimum detection level of bacterial growth was 10^3 colony forming units (c.f.u.) /g faeces. From each agar plate without antibiotics one colony with the appearance of *E. coli* was picked and tested for growth at 42 °C overnight in tryptone water (Oxoid L42) and for the indole reaction. If these tests were positive the microorganism was considered to be *E. coli*. For the first 50 isolates this identification was confirmed with Api-20E test (BioMérieux, Den Bosch, The Netherlands).

Prevalence of antibiotic resistance

The prevalence of antibiotic resistance was defined as the percentage of faecal samples showing any growth of *E. coli* on antibiotic-containing plates.

Degree of antibiotic resistance

The degree of resistance of each sample was calculated as the percentage of the total number of colonies that was resistant. Two degrees of antibiotic resistance to a particular antimicrobial agent were distinguished [10, 12], namely the proportion of faecal samples with a ratio < 50% was defined as low degree of resistance, and the proportion of faecal samples with a ratio ⩾ 50% was defined as high degree of resistance (thus the majority of the strains showed resistance to that agent).

The antimicrobial agents used in this study were selected because these or closely related compounds are regularly used for the treatment of humans and pigs in The Netherlands, except apramycin which is only registered for use in animals but is not extensively used in The Netherlands.

Statistical analysis

In the analysis of the differences in prevalence and degree of antibiotic resistance of the faecal samples of the three populations a Chi Square test with continuity correction was performed. A Fisher Exact test was used if the expected frequency in at least one cell was five or less. A two-sided significance level of $\leqslant 0{\cdot}05$ was used.

Multiple logistic regression was used to analyse the contribution of the origin of the three study populations (independent variable) to the prevalence of resistance (dependent variable) to a particular antibiotic. The antibiotics other than the dependent variable were considered to be independent variables simultaneously; a two-sided significance level of $\leqslant 0{\cdot}05$ was used.

The error of the method by using the spiral plater and by making tenfold dilutions, calculated from the standard error of the mean, was $0{\cdot}5\ \log_{10}$.

RESULTS

Ninety-five percent of the pigfarmer colonies, 94% of the abattoir worker colonies and 92% of the (sub)urban resident colonies that grew on Levine-agar showing the morphology typical of *E. coli* were identified as *E. coli*. The other colonies tested were also Enterobacteriaceae: *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp. and *Proteus* spp.

Finally, 278 samples of pigfarmers, 289 of abattoir workers and 150 of (sub)urban residents were included in the analysis. The other samples failed to grow on the agar plates without antibiotics.

Antibiotic use was recorded by 15 pigfarmers and 17 family members. Two farmers had been hospitalized recently. Twenty-five abattoir workers and 25 family members recorded antibiotic use, 5 abattoir workers had been hospitalized recently. By the (sub)urban residents no antibiotic use in the 3 previous months was recorded.

Intensive contact with pigs or pig carcasses was recorded by 182 abattoir workers, whereas 104 workers had other duties as well or no direct contact. No information about contact with pigs/carcasses was obtained from the remaining abattoir workers ($n = 30$). Fifty-two percent of the abattoir workers kept at least one domestic animal, whereas only three persons kept pigs.

Prevalence of antibiotic resistance

The prevalence and high degree of resistance are shown in Table 1 and Figure 1.

The most significant differences were noticed between pigfarmers and (sub)urban residents. The highest prevalence percentages were found for the pigfarmers and the lowest for the (sub)urban residents.

The highest percentages (i.e. 47%) in the abattoir workers and in the (sub)urban residents group were recorded for oxytetracycline and amoxicillin, respectively, and in the pigfarmer group for sulfamethoxazole (84%).

Further analysis as to the patterns of prevalence of resistance to amoxicillin, neomycin, oxytetracycline and trimethoprim of *E. coli* isolated from the three populations studied clearly showed that the highest percentage of fully susceptible strains (34%) as well as the lowest percentage of completely resistant isolates (4%) were found in the (sub)urban residents. The converse was observed for the pigfarmers.

Logistic regression analysis was performed to estimate the relative risk of prognostic and risk factors (i.e. antibiotics used and population groups) with

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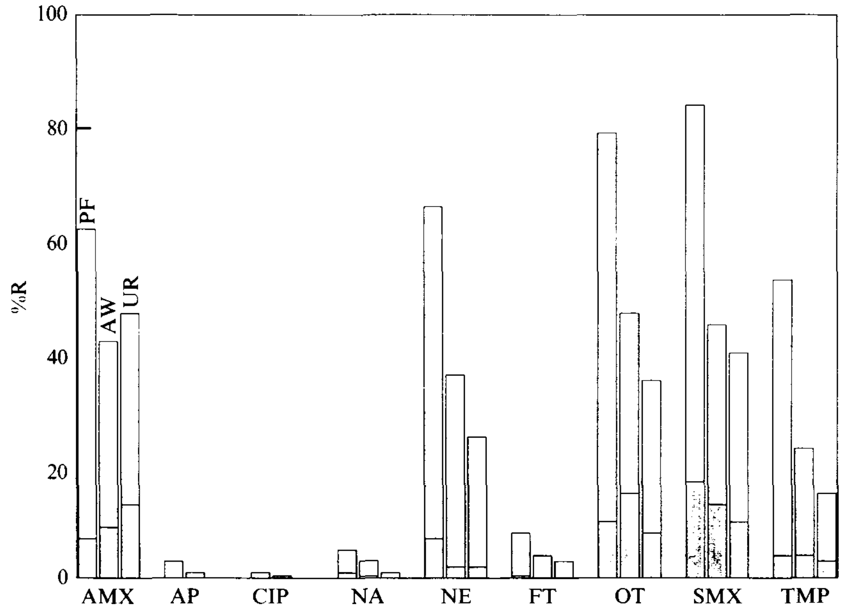

Fig. 1. Prevalence of antibiotic resistance (%) of *Escherichia coli* isolated from pigfarmers (PF, first bar per antibiotic), abattoir workers (AW, second bar) and (sub)urban residents (UR, third bar). Also shown are the proportions (%) of low degree (< 50%) ▧ and high degree (⩾ 50%) ■ of resistance. AMX, amoxicillin; AP, apramycin; CIP, ciprofloxacin; NA, nalidixic acid; NE, neomycin; FT, nitrofurantoin; OT, oxytetracycline; SMX, sulfamethoxazole; TMP, trimethoprim. %R, resistance percentage.

regard to the (sub)urban residents. The odds ratio (OR), with the 95% confidence interval (CI), for resistance to a particular antibiotic under consistent circumstances was calculated. Both pigfarmers (OR 0·4, CI 0·2–0·6) and abattoir workers (OR 0·5, CI 0·3–0·9) showed a low odds ratio for amoxicillin resistance. The pigfarmers showed a high odds ratio for neomycin (OR 3·6, CI 2·5–5·4), sulfamethoxazole (OR 6·5, CI 4·0–10·6) and trimethoprim (OR 2·1, CI 1·4–2·9). Resistance to oxytetracycline appeared to be independent of the population tested. For the other antibiotics tested no significant prognostic and risk factors were found.

Degree of antibiotic resistance

As presented in Figure 1 all three populations showed, except for neomycin, similar percentages for high degree of resistance, but distinct variations in low degree of resistance.

The prevalence and degree of resistance of the meat inspector samples were not significantly different from those of the abattoir workers. In addition, no differences in prevalence and degree of resistance were observed between abattoir workers with and without domestic animals. Because only three abattoir workers kept pigs no conclusions about the influence of regular contact with pigs could be drawn.

No significant differences could be observed between abattoir workers with intensive and those without or with less intensive contact with pig faecal contents or pig carcasses.

No significantly different prevalence or degree of resistance rates were observed for those people who had recently used antibiotics compared with those who had not used antibiotics recently (pigfarmers 5%, abattoir workers 8%). Nor were differences observed for those recording recent hospital stay (pigfarmers 1%, abattoir workers 2%) or antibiotic use by family members (pigfarmers 6%, abattoir workers 8%) when compared with those who did not record these factors.

DISCUSSION

The present study showed significant differences in prevalence of resistance between pigfarmers and (sub)urban residents for antibiotics extensively used in human and veterinary medicine in The Netherlands [25]. In contrast the prevalence of a high degree of resistance was, except for neomycin, not significantly different.

Several investigators have also observed differences in resistance of the faecal flora of pigfarmers/abattoir workers and urban residents [13, 26, 27] suggesting that contact with livestock was one route by which antibiotic resistance entered the human gut flora. In contrast, Levy and colleagues [12] found no significant difference between rural and urban residents. The general trend in their study was for lower numbers of resistant bacteria to be found in rural samples.

Remarkably, in the present study 15 (5%) pigfarmers and 25 (8%) abattoir workers used antibiotics during the 3 months previous to faecal sampling, whereas none of the (sub)urban residents mentioned recent antibiotic use. This might be an indication that people in contact with pigs or pig carcasses have a greater risk of bacterial infections. A recent study about occupational risk factors for pigfarmers showed that pigfarmers often suffer from chronic non-specific respiratory tract afflictions, because of regular exposure in pig-stables to dust containing fungi, endotoxins, disinfectants etc. [28]. This exposure results in a higher probability of respiratory tract infections, which could explain the relatively high percentage of antibiotic usage among the pigfarmers. Unfortunately, no information about the reasons of antibiotic therapy was obtained. Moreover, in one of the slaughter-houses studied, each month 4% of the workers were treated with antibiotics for wounds or eczema (personal communication). Because the control group of (sub)urban residents did not mention recent use of antibiotics, this therapeutic use among pigfarmers and abattoir workers might explain the higher prevalence of resistance in these groups than in the (sub)urban residents. The relatively low numbers of pigfarmers ($n = 15$) and abattoir workers ($n = 25$) who mentioned antibiotic use could not explain the observed differences between the three populations. Also recent use of antibiotics by family members appeared to be of no influence on antibiotic resistance in this study. Contact with pigs/carcasses and pig faeces might be a possible reason for the differences in prevalence of resistance observed between on one hand pigfarmers and abattoir workers and on the other hand the (sub)urban residents. Although no information about the professions of the last group was obtained, it is to be expected that they do not have regular direct contact with pigs. However, no significant differences were observed between the abattoir workers with intensive and those with less intensive pig contact. Therefore, other factors such as more intensive faecal contact, less personal hygiene and protection taken by farmers as compared to abattoir workers might have contributed to these differences. Moreover, it is very likely that direct contact with antibiotics used for treatment of pigs is an additional risk factor for emergence of resistance and selection of resistant strains in the intestinal flora of pigfarmers. The results of the logistic regression analysis underscore these suggestions.

Remarkably, significantly different prevalences and high degrees of resistance to neomycin were observed for the pigfarmers. Because neomycin is seldom used orally and never parenterally in human medicine but frequently in pigs, the suggestion seems likely that it is not human but mainly veterinary use of neomycin that is responsible for a higher prevalence and high degree of resistance in pigfarmers.

This study showed significant differences in the prevalence of antibiotic resistance in faecal *E. coli* of the three populations tested. Direct contact with pigs and pig carcasses may contribute to antibiotic resistance in pigfarmers and abattoir workers, in addition to common risk factors such as personal use of antibiotics. Moreover, it is likely that direct contact with antibiotics in medicated pig feed, i.e. mass medication, influences the prevalence of antibiotic resistance in pigfarmers. In the present study only prevalence and degree of resistance were determined. Comparison of plasmids and transfer experiments between pig and human isolates may elucidate the mechanisms involved.

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