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Antibiotic Resistance in Intestinal Commensal Bacteria Isolated from Faecal Samples from Pigs and Pig Farm Workers in Greece

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Abstract: The increased antibiotic resistance of intestinal commensal bacteria of food-producing animals in the last decade due to the extensive use of antibiotics is a potential risk for human health. In the present study, the prevalence of antibiotic resistance of *E. coli*, *E. faecalis* and *E. faecium* isolated from faecal samples from fattening pigs and pig farm workers who are not in contact with animals (group A) and pig farm workers who are in direct contact with animals (group B) was determined. The resistance of the bacteria was assessed by the determination of Minimum Inhibitory Concentration (MIC) of each antibiotic used in the study by microdilution method. *E. coli* isolated from fattening pigs showed resistance to at least one antibiotic used in the study at 93.20%, from pig farm workers of group A at 60.19% and of group B at 41.74%. The isolates of *E. faecalis* from fattening pigs, pig farm workers of group A and pig farm workers of group B were resistant to at least one antibiotic used in the study at 73.78, 68.93 and 52.42%. The resistance of *E. faecium* isolated from the same groups was determined at 62.13, 52.43 and 44.66%. *E. coli* and *Enterococci* isolated from pigs showed high resistance to tetracyclines, sulfamethoxazone, streptomycin and erythromycin, whereas those isolated from pig farm workers showed high resistance to tetracycline, ampicillin and erythromycin. The results of the study provide evidence that the use of antibiotics in pigs as well as the increased resistance of intestinal commensal bacteria affects the resistance of intestinal commensal bacteria in the persons working on the farms.

Key words: Antibiotic resistance, commensal bacteria, *E. coli*, *Enterococcus* sp.

INTRODUCTION

The discovery of antibiotics and their use for treating infections can be considered to be one of the most important milestones in the history of human medicine during the 20th century (Wright, 2007). Their use has dramatically improved the treatment of certain infectious diseases and prevented wound infections and their consequences.

Following their success in human medicine, antibiotics have been increasingly used to treat and prevent diseases in animals, fish and plants. Furthermore, sub-therapeutic doses of antibiotics have been intensively used as growth promoters in food-producing animals.

Shortly after the introduction of antibiotics into clinical use in human and veterinary medicine, as well as for enhancing animals' productivity, resistance of bacteria to these compounds began to emerge. Every time a new antimicrobial compound was used, its introduction was followed by the development of resistant bacteria (Levy, 1982). This is considered as the evolutionary response of bacteria in the presence of the selective pressure of the antimicrobial agent (Fraimow and Abrutyn, 1995). As a response to the presence of a single antimicrobial agent, resistance genes can reside in groups of 10 or more on plasmids or on self-transmissible transposons, permitting the selection of multi-drug resistance (Jacoby and Archer, 1991).

Taking into account the extremely short generation time of bacteria (minutes to hours), there are great opportunities for new mutations and the appearance of resistant genes, which are estimated to arise once in every 1 million to 1 billion cells and usually result in resistance to a single antibiotic (Khachatourians, 1998). Additionally, the abilities of bacteria for horizontal gene transfer played

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an important role in the wide dissemination of resistance genes, which can be transferred among bacteria through transduction, conjugation and transformation. Some of these mechanisms of horizontal gene transfer are limited to closely related bacterial species, whereas others can be accomplished between different species or even genera of bacteria (Jacoby and Archer, 1991).

Under these conditions, the extensive use and perhaps misuse of antibiotics has resulted in an increase in resistance of bacteria in human as well as veterinary origin (Aalbaek *et al*., 1991; Kayser, 1993; Mitsuhashi, 1993).

It is important to stress that antibiotic resistance is not only a characteristic of pathogenic bacteria. Resistance genes can be acquired or selected in commensal bacteria too. Although commensal bacteria are not pathogens, their role in disseminating resistance is important because they constitute a reservoir of resistance genes, which can be transferred to other bacteria including pathogens (Lukasova and Sustakova, 2003).

The transfer of resistance has been shown to occur between bacteria in humans, between bacteria of different animal species, as well as between bacteria from humans and animals and vice versa (Marshall *et al*., 1990; Shoemaker *et al*., 1992). Evidence is mounting that antibiotic-resistant enteric bacteria (*E. coli*, *Enterococci*) can be transferred from animals to humans via the food chain or by direct contact, leading to the establishment of a community reservoir of resistance genes (Van den Bogaard and Stobbering, 1999).

During the last decade, awareness has increased concerning the potential problems that could emerge on the human health front from antimicrobial-resistant bacteria among food-producing animals. Due to this, veterinary resistance monitoring systems have been established in many countries, which are based on determining the resistance of animal pathogens, zoonotic bacteria, as well as intestinal commensal bacteria like faecal *E. coli* and strains of *Enterococcus* sp. (Aarestrup, 2004).

Therefore, monitoring of intestinal commensal bacteria resistance gives a more representative estimation of the occurrence of resistance in the entire animal population and is considered a good indicator for selection pressure through antibiotic use on each animal's species and for resistance problems to be expected in pathogens (Lukasova and Sustakova, 2003).

The aim of the present study is to investigate and compare the prevalence of antibiotic resistance of intestinal commensal bacteria as *E. coli* and strains of *Enterococcus* sp. isolated from faecal samples taken from pigs and pig farm workers. The bacteria investigated in this study were isolated from faecal samples collected from fattening pigs at different farms and workers at the same farms with differing degrees of contact with animals and subsequently with animal faeces.

MATERIALS AND METHODS

Collection of faecal samples

Collection of samples from fattening pigs: Faecal samples were collected from fattening pigs at 112 pig farms sited in Central and Northern Greece. At each pig farm, 10 healthy fattening pigs 4 months of age were randomly selected. From each pig, a portion of faeces was removed from the rectum and placed in a sterile container. Sequentially, 1 g from each sample was transferred to another sterile container and all samples were mixed promptly so that a unique sample for each pig farm of 10 g of faeces was constituted.

Fattening pigs were chosen to be sampled because this group of pigs usually receives mass medication with antibiotics added into their food or water for treating or preventing diseases or for growth promotion. Additionally, these animals are stabled in groups close together, so any resistant bacteria can be transferred easily among the members of the group. Due to this, fattening pigs represent a more uniform population on the pig farm concerning the distribution of antibiotic resistant bacteria.

Collection of samples from pig farm workers: On each pig farm, one worker who worked in the animal food processing sector and was not in direct contact with animals (group A) and one worker who worked in the animal houses and was in close contact with animals daily (group B) were asked to collect a small portion of their faeces in a sterile plastic container. Faecal samples were collected from workers who declared that they are not under treatment with antibiotics for any disease and also had not received any treatment based on antibiotics in the last 4 months. Each sample was coded so the confidentiality of the workers' data could be respected and assured.

Isolation and identification of intestinal commensal bacteria: For the isolation of *E. coli* and *Enterococcus* sp., 0.5 g of faeces was diluted to 4.5 mL of Phosphate Buffer Saline (PBS, pH 7.2) (Sigma-Aldrich Co.) so that a suspension 1:10 w v^{-1} was created. The suspension was filtered through sterile gauze in a sterile container in order to remove any solid material. Sequentially, 0.1 mL of the filtered suspension was spread on MacConkey and

Slanetz-Bartley agar (Biolife Italiana s.r.l) plates for isolating *E. coli* and strains of *Enterococcus* sp., respectively. The MacConkey agar plates were incubated overnight at 37°C, whereas the Slanetz-Bartley agar plates were incubated at 37°C for 48 h.

One lactose-positive colony with the typical morphology for *E. coli* was selected from every MacConkey agar plate and subcultured on blood agar (10% bovine blood). After overnight incubation at 37°C, the isolates were tested for tryptophanase and β-glucuronidase production using a double test tablet (DIATABS™) for β-glucuronidase (PGUA) and indole test (ROSCO Diagnostica A/S). Only strains showing positive reactions in both tests were selected for further antibiotic susceptibility testing.

Different colonies randomly selected from every Slanetz-Bartley agar plate were subcultured on bile-aesculin and blood agar (Biolife Italiana s.r.l). Colonies morphologically consistent to enterococci and catalase negative with positive reaction to bile-aesculin agar were selected. The strains *E. faecalis* and *E. faecium* were identified on the basis of the results of biochemical tests for fermentation of arabinose, mannitol, sorbitol, sorbose and lactose. The biochemical tests were selected from the panel of biochemical tests proposed for identifying *Enterococcus* sp. strains (Manero and Blanch, 1999; Day *et al*., 2001).

Antibiotic susceptibility testing: The susceptibility of *E. coli* was assessed for Ampicillin (AM), Tetracycline (TE), Chloramphenicol (CHL), Gentamycin (GE), Trimethoprim (TRI), Sulfamethoxazole (SUL), Streptomycin (STR), Neomycin (NE), Ceftiofur (CEF), Enrofloxacin (ENR) and Nalidixic Acid (NAL). The susceptibility of *Enterococcus* sp. was determined for ampicillin, tetracycline, chloramphenicol, gentamycin, streptomycin, neomycin, Erythromycine (ER), Vancomycin (VAN) and Virginiamycin (VIRG) (Sigma-Aldrich Co). The antibiotics were supplied as powders and the stock solutions were created by diluting each one with the solvent and diluent recommended by the manufacturer, taking into account the potency of each antibiotic base. The stock solutions were aliquoted in 1000 μL volume and stored at -70°C until use (NCCLS, 2003).

The susceptibility of the isolated bacteria was assessed by definition of Minimum Inhibition Concentration (MIC) for each antibiotic used in the study by broth microdilution method performed in 96 round bottom well microplates at a volume of 0.1 mL, as it is described by NCCLS (now named CLSI) (NCCLS, 2003).

Initially, a series of two-fold dilutions were prepared for each antimicrobial agent in the microplate, diluting properly the stock solution in Mueller-Hindon broth with adjusted cations (Difco®). In each microplate well, 50 μL of the antimicrobial solution was added. The concentration of antimicrobial agent in this solution was double that of the final amount wanted because after the addition of equal volume (50 μL) of bacterium inoculum suspension, the antimicrobial solution would be further diluted (1:2 dilution). In each microplate, two wells were left as controls in which 50 μL of Mueller-Hindon broth was placed instead of antimicrobial solution.

For preparing bacteria inoculants, suspensions for every bacterium were created in Mueller-Hindon broth with a concentration of $1\text{-}2\times10^8$ CFU mL^{-1}. Bacteria concentration in each suspension was determined by visual comparison with 0.5 MacFarland's standard against a white background with contrasting black lines. The inoculums were further diluted 1:100 by adding Mueller-Hindon broth, so suspensions with a concentration of 10^6 CFU mL^{-1} were prepared. From these inoculums, 50 μL was added to each well in the microplate (including controls) and mixed with the antimicrobial's agent suspension, resulting in a final concentration of 5×10^5 CFU mL^{-1}.

After the addition of bacteria inoculum, the microplates were sealed with a self-adhering plastic film in order to avoid evaporation and incubated aerobically at 35°C for 18 h.

When the incubation was complete, the microplates were removed from the incubator and the results read by placing the microplate on a viewing device with an enlarging mirror. A bench lamp providing indirect light facilitated reading. Bacterial growth was easily detected in the mirror as a pellet at the bottom of the well.

The MIC for each antimicrobial agent was determined as the lowest concentration completely inhibiting visible growth of the bacterium tested.

For quality control, the reference strains of *E. coli* ATCC 25922 and *E. faecalis* ATCC 29212 were used.

Data analysis: The collected data were analyzed and the proportions compared by chi-square test using Medcalc version 8.0 for Windows (Schoonjans *et al*., 1995).

RESULTS

If for any reason *E. coli*, *E. faecalis* and *E. faecium* were not isolated from faecal samples collected from fattening pigs and pig farm workers from both groups (group A and group B) from a pig farm, then the samples collected from this farm were discarded. Under these conditions, 103 *E. coli*, *E. faecalis* and *E. faecium* were isolated from fattening pigs as well as from pig farm

Table 1: Antibiotic resistance of *E. coli* isolated from fattening pigs and pig farm workers which are not in contact with animals (group A) and with direct contact with animals (group B) n = 103

		Resistant *E. coli* isolated from fattening pigs and pig farm workers					
Antimicrobial agent break point mg L^{-1}		Fattening pigs n = 96		Pig farm workers of group A n = 62		Pig farm workers of group B n = 43	
		Nr *	% of resistant**	Nr*	% of resistant**	Nr*	% of resistant**
Ampicillin	>8	18	18.75	21	33.87	17	39.53
Ceftiofur	>2	1	1.04	3	4.84	2	4.65
Chloramphenicol	>16	9	9.38	5	8.06	3	6.98
Enrofloxacin	>0.25	6	6.25	3	4.84	3	6.98
Gentamicin	>8	2	2.08	2	3.23	1	2.33
Nalidixic acid	>16	8	8.33	4	6.45	4	9.30
Neomycin	>8	4	4.17	4	6.45	2	4.65
Streptomycin	>32	11	11.46	8	12.90	5	11.63
Sulfamethoxazone	>256	32	33.33	12	19.35	8	18.60
Tetracycline	>8	63	65.63	43	69.35	30	69.77
Trimethoprim	>8	25	26.04	5	8.06	5	11.63

*The number of resistant bacteria reported is greater than the resistant isolates because many bacteria shown multi-drug resistance, **The percentages are calculated on the basis of resistant strains

Table 2: Antibiotic resistance of *E. faecalis* isolated from fattening pigs and pig farm workers which are not in contact with animals (group A) and with direct contact with animals (group B) n = 103

		Resistant *E. faecalis* isolated from fattening pigs and pig farm workers					
Antimicrobial agent break point mg L^{-1}		Fattening pigs n = 76		Pig farm workers of group A n = 71		Pig farm workers of group B n = 54	
		Nr**	% of resistant***	Nr**	% of resistant***	Nr**	% of resistant***
Ampicillin	>8	13	17.10	20	29.41	18	34.61
Chloramphenicol	>16	8	10.52	4	5.88	3	5.76
Erythromycine	>4	13	17.10	19	27.94	13	25.00
Gentamycin	>512	6	7.89	6	8.82	5	9.61
Neomycin	>1024	9	11.84	4	5.88	6	11.53
Streptomycin	>1024	15	19.73	12	17.64	9	17.30
Tetracycline	>8	61	80.26	37	54.41	35	67.30
Vancomycin	>16	0	0	0	0	0	0
Virgianamycin	>8	*		*		*	

* Not applicamble for *E. faecalis*, **The number of resistant bacteria reported is greater than the resistant isolates because many bacteria shown multi-drug resistance, ***The percentages are calculated on the basis of resistant strains

workers. All the workers from whom faecal samples were collected were males and their ages ranged from 35-62 years.

The strains of *E. coli* isolated from fattening pigs, were resistant at least to one antibiotic used in the study at 93.20% (96 out of 103). Those isolated from pig farm workers of group A showed resistance at 60.19% (62 out of 103) and those of group B at 41.74% (43 out of 103), respectively. The percentage of resistant *E. coli* strains isolated from fattening pigs was greater and differed significantly ($p<0.05$) from those isolated from pig farm workers in both groups. However, the percentage of resistant *E. coli* strains isolated from pig farm workers in group A (not in direct contact with animals) was greater and differed significantly ($p<0.05$) from that of *E. coli* isolated for farm workers in group B (in direct contact with animals).

The number and percentages of resistant *E. coli* isolated from fattening pigs as well as from farm workers of group A and B according to their resistance in each antibiotic used in this study are presented in Table 1.

From the strains of *E. coli* isolated from fattening pigs, 48.95% (47 out of 96) showed resistance to 2 or more antibiotics, whereas 37.09% (23 out of 62) and 34.88% (15 out of 43) of those isolated from pig farm workers of group A and group B showed multi-drug resistance, respectively. Although the percentages of multi-drug resistant *E. coli* isolated from fattening pigs, pig farm workers of group A and group B differ, the difference is not significant ($p>0.05$).

The strains of *E. coli* isolated from faecal samples coming from fattening pigs showed resistance mainly to tetracycline, sulfamethoxazone, trimethoprim and ampicillin. On the other hand, the strains of *E. coli* isolated from faecal samples coming from pig farm workers of both groups showed greater resistance to tetracycline and ampicillin. The percentage of ampicillin-resistant *E. coli* isolated from pig farm workers was greater and differed significantly ($p<0.05$) from that of resistant strains isolated from fattening pigs.

The number and percentage of resistant *E. faecalis* and *E. faecium* isolated from faecal samples collected from fattening pigs as well as from pig farm workers of group A and group B according to antibiotics used in this study are presented in Table 2 and 3.

Table 3: Antibiotic resistance of *E. faecium* isolated from fattening pigs and pig farm workers which are not in contact with animals (group A) and with direct contact with animals (group B) n = 103

		Resistant *E. faecium* isolated from fattening pigs and pig farm workers					
Antimicrobial agent break point mg L^{-1}		Fattening pigs n = 64		Pig farm workers of group A n = 54		Pig farm workers of group B n = 46	
		Nr*	% of resistant**	Nr*	% of resistant**	Nr*	% of resistant**
Ampicillin	>8	9	14.06	14	25.92	14	30.43
Chloramphenicol	>16	4	6.25	3	5.55	3	6.52
Erythromycine	>4	15	23.43	11	20.37	12	26.08
Gentamycin	>512	9	14.06	6	11.11	6	13.04
Neomycin	>1024	6	9.37	4	7.40	4	8.69
Streptomycin	>1024	12	18.75	7	12.96	8	17.39
Tetracycline	>8	51	79.68	38	70.37	32	69.56
Vancomycin	>16	0	0	0	0	0	0
Virgianamycin	>8	4	6.25	2	3.70	2	4.34

* The number of resistant bacteria reported is greater than the resistant isolates because many bacteria shown multi-drug resistance, **The percentages are calculated on the basis of resistant strains

The isolates of *E. faecalis* from fattening pigs, pig farm workers of group A and pig farm workers of group B are resistant at least to one antibiotic used in the study at 73.78% (76 out of 103), 68.93% (71 out of 103) and 52.42% (54 out of 103), respectively. The resistance of *E. faecium* isolated from the same groups was determined at 62.13% (64 out of 103), 52.43% (54 out of 103) and 44.66% (46 out of 103), respectively.

The percentage of multi-drug resistance for *E. faecalis* and *E. faecium* isolated from fattening pigs was determined at 28.94 and 39.06%, which do not differ significantly ($p>0.05$), as chi-square test reveals. The percentages of multi-drug resistance for *E. faecalis* and *E. faecium* isolated from pig farm workers of group A was determined at 28.16 and 35.19% and from pig farm workers of group B at 33.33 and 39.13%, respectively. Vancomycin-resistant *Enterococcus* sp. was not isolated from fattening pigs and from pig farm workers in both groups in the present study.

The percentages of ampicillin-resistant strains of *E. faecalis* and *E. faecium* isolated from pig farm workers in both groups were greater than that of the strains isolated from fattening pigs, although the difference was not significant ($p>0.05$). Additionally, it must be noted that the vast majority of *E. faecalis* and *E. faecium* isolated from fattening pigs showing resistance to erythromycine were multi-drug resistant strains.

DISCUSSION

The antibiotic resistance of intestinal commensal bacteria isolated from faecal samples collected from fattening pigs, pig farm workers preparing animals' food but not in direct contact with animals and pig farm workers working in animal houses in direct contact with animals was determined and compared.

The antibiotic resistance of *E. coli*, *E. faecalis* and *E. faecium* isolated from fattening pigs was higher than that determined for the same bacteria isolated from pig farm workers in both groups. The high prevalence of antibiotic resistance found in intestinal commensal bacteria isolated from fattening pigs in Greece is in agreement with those found in other EU member states where veterinary antimicrobial resistance monitoring systems exist (ITAVARM, 2003; SVARM, 2004).

The increased resistance of intestinal commensal bacteria isolated from fattening pig faeces must be attributed to the intensification of pig production worldwide and the extensive use of antibiotics for treating and preventing animal diseases, as well as for enhancing productivity. It is documented that the level of exposure to antibiotics creates favorable conditions for increasing bacteria's antibiotic resistance and affects its extension, too (Sundle *et al*., 1998). Additionally, the population density is a crucial factor for the dissemination of resistant bacteria and resistant genes between individuals, contributing to further increase of antibiotic resistance in a population (Bruinsma *et al*., 2003).

The vast majority of intestinal commensal bacteria isolated from fattening pigs showed resistance to tetracyclines, whereas the strains of *E. coli* also showed increased resistance to sulfamethoxazone and trimethoprim. On the other hand, the strains of *Enterococcus* sp. showed increased resistance to erythromycin and streptomycin.

The widespread resistance of intestinal commensal bacteria to tetracyclines can be attributed to the extensive and long-term use of this antibiotic for veterinary therapy, prophylaxis and as growth promoter (especially in pigs), resulting in the selection of resistant pathogenic and commensal bacteria (Khachatourians, 1998). According to many studies results, the majority of commensal and pathogenic bacteria in the past were susceptible to tetracyclines, but resistance has emerged due to genetic acquisition of *tet* genes, which encode the resistance mechanisms based on efflux pumps and ribosomal protection proteins (Chopra and Roberts, 2001).

The increased resistance of strains of *E. coli* to sulfamethoxazone and trimethoprim must be connected to the fact that the combination of these antibacterial compounds was used until recently for controlling respiratory diseases, administered to pigs though food. This contributed to an increase in the resistant bacteria population in this animal species because for a long time there was a selective pressure for emerging resistant clones that already pre-existed in the bacteria population (Corpet *et al*., 1989).

A significant portion of *E. coli* and *Enterococcus* sp. isolates from fattening pigs showed resistance to chloramphenicol. Note that all strains resistant to chloramphenicol showed multiple resistance to more than three antibiotics tested in the study. The existing resistance to chloramphenicol, although this compound has not been used in veterinary practice for the last 17 years, can be ascribed to the use of other antibiotics, even from different groups and different molecular structures. There is evidence that some resistance to an antibiotic may persist long after its use has been banned because the use of another antibacterial substance can select for resistance to that (co-selection) due to the two resistance determinants being genetically linked on the same plasmid or transposon (Phillips *et al*., 2004).

The *Enterococcus* sp. isolates from fattening pigs showed considerable resistance to erythromycin, even if this antibiotic is not widely used in pigs. The widespread resistance of *Enterococcus* sp. to macrolide antibiotics can be traced to the widespread use of other antibiotics belonging to the same group. This can be seen especially with tylosin, which is used commonly in normal doses for treating respiratory diseases in pigs. Additionally, tylosin was also used until recently in subtherapeutic doses in pigs as a food additive for prophylaxis from respiratory diseases. This is supported from the findings of studies concerning the mechanisms of resistance to macrolides, which reveal that when the mechanism of resistance is based on modification of drug target, the single alteration of the 23S rRNA confers broad cross-resistance to macrolide-lincosamine-streptogramin antibiotics (Portillo *et al*., 2000). The resistance of *E. faecium* to virginiamycin observed in the present study must also be attributed to this phenomenon of cross-resistance.

The results of the present study reveal that the *Enterococcus* sp. isolated from fattening pigs express a considerable level of resistance to aminoglycosides as well as to ampicillin. It must be pointed out that neomycin is the antibiotic commonly used in pig farming as a food additive for preventing enteritis in young pigs. Resistance to one antibiotic of the aminoglycoside group very often results in resistance to other members of the group, although they are not used for treating or preventing diseases in a population (Donabedian *et al*., 2003). Concerning ampicillin resistance, it must be pointed out that *Enterococci* have intrinsic resistance to cephalosporins and are able to develop widespread resistance to penicillin and ampicillin (Jeljaszewicz *et al*., 2000).

The results of the present study reveal a considerable level of resistance of intestinal commensal bacteria isolated from pig farm workers independent of the level of their contact with animals. It is documented that on farms where the animals have high prevalence and degree of resistance in their intestinal flora the workers' intestinal commensal bacteria show high prevalence of resistance, too. This suggests that working in an environment with resistant bacteria poses a risk for acquiring resistant bacteria (Bruinsma *et al*., 2003). The results of many studies indicate that resistant commensal bacteria can be spread from animal to humans and vice versa (Barton, 2000). Note that in many cases, animal-adapted intestinal commensal bacteria are not able to colonize in human intestines and they are recovered only for a transient period from the recipient's faeces. Even in such cases, resistant bacteria could transfer resistance plasmids to commensal bacteria in human intestines (Shoemaker *et al*., 1992).

The resistance of *E. coli* and *Enterococcus* sp. isolated from pig farm workers who are not in direct contact with animals and working in animals' food preparation (group A) is higher than that determined for the same bacteria isolated from pig farm workers who are in direct contact with animals and working in animal houses (group B), although the difference for *Enterococci* is not significant. The difference in the prevalence of resistance can be associated with the different working conditions for the two groups. The workers preparing the food for animals are in direct contact and for a considerable amount of time every day handle antibiotics used as feed additives for treatment or prevention. Under these conditions and taking into account that workers usually do not take preventive measures, they can ingest through hand contamination or even inhale amounts of antibiotics, which contribute to the development of resistant bacteria. On the contrary, workers who are in direct contact with animals usually obtain resistant bacteria though contact with animal faeces due to the unsanitary conditions in which they are working.

Taking into account that the main factor for developing resistance by bacteria is the selective pressure of antibiotics, it can be assumed that the resistance of bacteria isolated from persons who are in direct contact with antibiotics without taking preventive measures would be higher than that of bacteria isolated from persons who are in close contact with animals.

The vast majority of *E. coli* and *Enterococcus* sp. isolated from pig farm workers in both groups showed

resistance to tetracyclines, whereas *E. coli* showed higher resistance to ampicillin compared to strains isolated from fattening pigs. This can be attributed to the fact that tetracyclines are widely used in human medicine as well as veterinary medicine. The high resistance of *E. coli* to ampicillin found in this study is in agreement with the results of the study conducted by Bruinsma *et al*., 2003, who determined the resistance of *E. coli* in faecal samples collected from healthy inhabitants in Athens, Greece. In this study, it was found that *Enterococci* isolated from healthy persons living in Athens, Greece, also showed high resistance to erythromycin. The high erythromycin resistance of *Enterococci* isolated from pig farm workers can be attributed to the fact that this antibiotic is widely used in human medicine and in most cases is the antibiotic of choice of many physicians for treating upper respiratory tract infections.

It must be pointed out that in the present study vancomycin-resistant *Enterococci* were not found. This must be associated with the fact that the use of avoparcin, which shows cross-resistance to vancomycin, was banned by the European Union (EU) 10 years ago (1997) and that this compound was used mainly in poultry, whereas its use in pigs was very limited in Greece.

CONCLUSION

The results of the present study reveal that a considerable resistance has developed to intestinal commensal bacteria in fattening pigs due to the use of antibiotics in veterinary practice in Greece.

There is evidence that the use of antibiotics in pigs as well as the increased resistance of intestinal commensal bacteria affects the resistance of intestinal commensal bacteria in the persons working on the farms. Further research is required on this issue so that the way antibiotic resistance is developed in the intestinal bacteria of persons working in farm environments can be explored and documented.

Taking into account the effect antimicrobial resistance has on human health and its economic impact, measures to delay the development of resistance are urgently needed. This includes judicious use of antibiotics in veterinary practice and food animal rearing and utilizing control measures to decrease resistance in reservoirs on farms and in the environment.

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