

Resistance in *Escherichia coli* Strains Isolated from Pig Faecal Samples and Pig farm Workers, Greece

¹George Valiakos, ²Alexandros Vontas, ¹Constantina N. Tsokana,
¹Alexios Giannakopoulos, ¹Dimitrios Chatzopoulos and ¹Charalambos Billinis

¹Faculty of Veterinary Science, University of Thessaly, 224 Trikalon Street, Karditsa, Greece

²Department of Medical Laboratories, Technological Educational Institute of Thessaly, Larissa, Greece

Article history

Received: 22-09-2016

Revised: 01-11-2016

Accepted: 05-11-2016

Corresponding Author:

George Valiakos
Faculty of Veterinary Science,
University of Thessaly, 224
Trikalon Street, Karditsa,
Greece
Tel: +3024410 66011
Email: georgevaliakos@gmail.com

Abstract: In this study, we compare the degree of antibiotic resistance between the *E. coli* strains isolated from swine faecal and farm workers. Resistance in nine widely used antibiotics was assessed by the determination of Minimum Inhibitory Concentration. Sixty-four out of 72 *E. coli* isolates derived from pigs and sixty out of 72 derived from farm workers showed resistance to at least one antibiotic. High resistance in tetracycline, ampicillin and amoxicillin was detected in both human and swine *E. coli* isolates, showing similar resistance patterns. This finding indicates that the use of antibiotics in pig industry and the increased antibiotic resistance of animal *E. coli* isolates, also affects the resistance of isolates collected from farm workers, at least in the area of study.

Keywords: Antibiotic Resistance, *E. coli*, Greece, Minimum Inhibition Concentration, Pigs

Introduction

Antibiotic resistance, driven by the extensive use of antibiotics, is considered to be one the major future threats regarding effective treatment of bacterial diseases. Resistant bacteria are favoured by selection pressure during use of antibiotics and thus are spread in animals and humans raising concerns regarding prevention and treatment (Osterberg *et al.*, 2016). Additionally, the uncontrolled use of antibiotics in food-producing animals is worrying not only from a veterinary clinical perspective but from a zoonotic aspect as well (Marshall and Levy, 2011). In pig industry, antibiotics are used extensively in three ways-as growth promoters, as prophylactic or metaphylactic treatment and for therapeutic purposes (Barton, 2014).

The aim of this study was to investigate the prevalence of antibiotic resistance in faecal *E. coli* isolates from healthy pig farm workers and compare these data with isolates obtained from pigs in the same farms.

Materials and Methods

The investigation was performed between August and December 2015 in pig farms in the region of Thessaly, central Greece. A mixed fresh faecal sample was randomly collected from four healthy fattening pigs with the permission of the pig farmer. Use of antibiotics

in this class of pigs is common and they live in close contact, allowing the easy transfer of resistant bacteria among them. A fresh faecal sample was collected from one of the pig workers in each pig farm. Faecal samples were collected only from apparently healthy workers that had not received any antibiotic treatment in the last 3 months. The faecal samples were collected in sterile plastic containers and they were sent immediately to the University of Thessaly where they were kept refrigerated until analysis within one to four days after sampling.

In brief, 1 g of each sample was diluted to 9 mL of PBS pH 7,2 to create a suspension of 1:10 w/v and was filtered in order to remove all solid material. A quantity of 0,1 mL of the suspension was plated onto MacConkey agar. The agar plates were incubated at 37°C for 24 h. Candidate colonies were then plated in trypticase soy agar medium and biochemically characterized using the API20E system (Biomérieux, France).

If *E. coli* was not isolated from faecal samples collected from both pigs and workers from a pig farm, samples collected from this farm were discarded and not included in the analysis. Under these conditions, from a total of 86 pig farms investigated, a total of 114 *E. coli* were isolated from pigs as well as from workers from the same farms (72 of each group from 72 farms). These *E. coli* isolates were selected for the purpose of determining susceptibility.

Table 1. Antibiotic resistance of *E. coli* isolated from pigs and pig farm workers

Antimicrobial agent	Break point mg/L	Resistant <i>E. coli</i> isolated from pigs and workers				Farms with resistance in both pigs and workers/total resistance positive farms
		Pigs n = 72		Workers n = 72		
		N	% resistance	N	% resistance	
Amoxicillin	>8	36	50.0	33	45.8	32/37 (86%)
Ampicillin	>8	28	38.9	30	41.7	28/30 (93%)
Cefotaxime	>2	0	0.0	0	0.0	0/0 (0%)
Trimeth/sulfam	>4	12	16.7	6	8.3	6/12 (50%)
Nalidixic Acid	>16	12	16.7	6	8.3	6/12 (50%)
Neomycin	>1024	4	5.6	3	4.1	3/4 (75%)
Tetracyclin	>8	36	50.0	27	37.5	26/37 (70%)
Gentamycin	>512	0	0.0	3	4.2	0/0 (0%)
Chloramphenicol	>16	4	5.6	9	12.5	4/9 (44%)

Resistance was assessed for Amoxicillin, Ampicillin, Cefotaxime, Trimethoprim-Sulfamethoxazole, Nalidixic Acid, Neomycin, Tetracyclin, Gentamycin and Chloramphenicol. Antibiotics were supplied as powders and each one was diluted as recommended by the manufacturer.

The Minimum Inhibition Concentration was determined for each sample and each antibiotic by broth microdilution performed in 96 round bottom well microplates, following the standards of the Clinical and Laboratory Standards Institute (CLSI, 2013; Minas *et al.*, 2008).

Results

Table 1 presents the number and percentages of *E. coli* isolates from pigs and pig workers that were found resistant to the antibiotics used in this study. Of isolates from pigs, 64 (88,8%) were found resistant to at least one antimicrobial agent and 36 (50%) to at least two agents. Of isolates from pig workers, 60 (83,3%) were found resistant to at least one antimicrobial agent and 27 (37.5%) to at least two agents. There were 28 farms that isolates from pigs were resistant to only one antibiotic and 33 farms that isolates from workers were also resistant to only one antibiotic; in 25 of them both isolates were resistant to the same antibiotic and in 11 more there was a different resistance in the pig and the human isolate (3 and 8 respectively). No statistically significant difference was found between the resistance percentages ($\chi^2 = 0.929$, $df = 1$, $p = 0.33$) neither between the multi-drug resistance percentages ($\chi^2 = 2.286$, $df = 1$, $p = 0.13$) of pig and pig workers isolates. In most resistance-positive farms, isolates of both pigs and workers were resistant in the same antibiotics (Table 1). Resistance to tetracycline, amoxicillin and ampicillin was detected in *E. coli* isolates in the highest frequency from pigs and pig workers.

Discussion

The most frequent pattern of antimicrobial resistance found in both human and pig *E. coli* isolates is resistance to

ampicillin, amoxicillin and tetracycline. Similar patterns have been found in other studies as well (Alali *et al.*, 2008; Minas *et al.*, 2008). Due to their relatively low cost and high availability for sale ‘over the counter’, these drugs are widely used by farmers for therapeutic and prophylactic applications (Glass-Kaasta *et al.*, 2013). Callens and co-workers in Belgium, where prudent use guidelines have not been implemented, reported that antibiotics used included some important human antimicrobials such as amoxicillin (Callens *et al.*, 2012).

Recent studies have reported that swine farm workers were at higher risk of exhibiting multidrug-resistant *E. coli* or showed higher antibiotic resistances than non-swine workers (Alali *et al.*, 2008; Cho *et al.*, 2012). These facts are possibly due to the use of several antimicrobial agents at swine farms and the intensive farm management practices on swine farms that may cause the transmission of antibiotic resistant bacteria in both swine hosts and farm environment. In our study, similar resistances are demonstrated in both swine farmers and animals. An explanation for this is that resistant bacteria may be readily transferred from food animals to humans because these bacteria may colonize humans via contact through occupational exposure, or waste runoff from animal production facilities. A systematic review has concluded that oral use of antibiotics in animals increases the prevalence of antibiotic resistant *E. coli* in treated pigs and by extension the risk of transfer of this resistance to humans (Burow *et al.*, 2014). In Greece, relevant regulations are harmonized with European Union (EU) policies: EU banned the use of all antimicrobial as growth promoters, so as to reduce antimicrobial resistance traits in the microbial flora of farm animals (Maron *et al.*, 2013). However, in many cases use of antibiotics is being continued for “therapeutic” reasons, highlighting the importance of clearly defining “therapeutic” and “non therapeutic” use.

Conclusion

Data obtained in our study indicate that usage of antibiotics in animals may contribute to the prevalence

of resistance in *E. coli* human strains as well in a similar pattern. The microbial ecosystem of humans, animals and food are undoubtedly connected in a large extend and the risk of transfer of antimicrobial resistant bacteria to humans should be considered high. It is, thus, recommended that the use of antimicrobial agents in food animals should be limited wherever possible to minimize the selection and spread of resistant bacteria and that farm management practices and hygiene should be improved to minimize the risk of transfer of antimicrobial resistant bacteria to humans.

Acknowledgement

The authors wish to thank the farmers and herd keepers to allow us the access.

Funding Information

The authors have no support or funding to report.

Author's Contributions

George Valiakos: Designed the study, conducted the experiments, analysed data and contributed to writing the manuscript.

Alexandros Vontas: Performed human samples collection contributed to data interpretation and writing of the manuscript.

Constantina N. Tsokana: Conducted experiments and reviewed the manuscript

Alexios Giannakopoulos: Performed animal samples collection, reviewed the manuscript.

Dimitrios Chatzopoulos: Conducted experiments and reviewed the manuscript.

Charalambos Billinis: Critical revision of the manuscript.

Conflict of Interest

The Authors declare that there is no conflict of interest.

References

Alali, W.Q., H.M. Scott, R.B. Harvey, B. Norby and D.B. Lawhorn *et al.*, 2008. Longitudinal study of antimicrobial resistance among *Escherichia coli* isolates from integrated multisite cohorts of humans and swine. *Applied Environ. Microbiol.*, 74: 3672-3681.
DOI: 10.1128/AEM.02624-07

Barton, M.D., 2014. Impact of antibiotic use in the swine industry. *Curr. Opin. Microbiol.*, 19: 9-15.
DOI: 10.1016/j.mib.2014.05.017

Burow, E., C. Simoneit, B.A. Tenhagen and A. Käsbohrer, 2014. Oral antimicrobials increase antimicrobial resistance in porcine *E. coli*-a systematic review. *Prev. Vet. Med.*, 113: 364-375.
DOI: 10.1016/j.prevetmed.2013.12.007

Callens, B., D. Persoons, D. Maes, M. Laanen and M. Postma *et al.*, 2012. Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. *Prev. Vet. Med.*, 106: 53-62.
DOI: 10.1016/j.prevetmed.2012.03.001

Cho, S.H., Y.S. Lim and Y.H. Kang, 2012. Comparison of antimicrobial resistance in *Escherichia coli* strains isolated from healthy poultry and swine farm workers using antibiotics in Korea. *Osong Public Health Res. Perspect.*, 3: 151-155.
DOI: 10.1016/j.phrp.2012.07.002

CLSI, 2013. VET01-A4: Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Fourth Edition. Clinical and Laboratory Standards Institute, USA.

Glass-Kaasta, S.K., D.L. Pearl, R.J. Reid-Smith, B. McEwen and S.A. McEwen *et al.*, 2013. Describing antimicrobial use and reported treatment efficacy in Ontario swine using the Ontario swine veterinary-based Surveillance program. *BMC Vet. Res.*, 9: 238-245. PMID: 24289212

Maron, D.F., T.J.S. Smith and K.E. Nachman, 2013. Restrictions on antimicrobial use in food animal production: An international regulatory and economic survey. *Globalizat. Health*, 9: 48-48.
DOI: 10.1186/1744-8603-9-48

Marshall, B.M. and S.B. Levy, 2011. Food animals and antimicrobials: Impacts on human health. *Clin. Microbiol. Rev.*, 24: 718-733.
DOI: 10.1128/CMR.00002-11

Minas, A., E. Petridou, E. Bourtzi-Chatzopoulou, V. Krikelis and A. Papaioannou, 2008. Antibiotic resistance in intestinal commensal bacteria isolated from faecal samples from pigs and pig farm workers in Greece. *Res. J. Biol. Sci.*, 3: 193-200.

Osterberg, J., A. Wingstrand, A. Nygaard Jensen, A. Kerouanton and V. Cibir *et al.*, 2016. Antibiotic resistance in *Escherichia coli* from pigs in organic and conventional farming in four European countries. *PloS One*, 11: e0157049-e0157049.
DOI: 10.1371/journal.pone.0157049