41. Antimicrobial resistance in Portuguese swine production – 2013 and 2014
Laboratory data
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Abstract

Antimicrobial resistance is a growing concern for both human and animal health, as well as the environment. Several countries established surveillance programs for antimicrobial use in animals and in Portugal we also have the PANRUAA, since 2014, that encourages antimicrobial use reduction in animals. As swine production is one of the food industries where the antimicrobial use deserves attention, we collected laboratory data from pig samples to obtain information on antimicrobial resistance in Portuguese swine production. Escherichia coli is an indicator bacteria of normal intestinal flora of swine and in this study, we used Escherichia coli isolates to analyze antimicrobial resistance. Our aim was to contribute to the knowledge of antimicrobial resistance frequencies in Portuguese swine farms.

Seventy seven strains of Escherichia coli were isolated from intestinal swabs or stools of swine with symptoms of enteric pathology. Those samples came from 26 different farms and antimicrobial resistance was evaluated according to CLSI guidelines, by diffusion disc method.

All Escherichia coli strains were resistant to more than one antimicrobial. Lincomycin, Doxycycline, Amoxycillin, Ampicillin and Oxytetracycline were the antimicrobials with the highest frequency of resistant strains. The lowest frequencies of antimicrobials resistant strains were found for Neomycin, Gentamicin, Ceftiofur and Marbofloxacin. Comparing antimicrobials resistance frequencies from both years, we found a significant increase for Amoxycillin, Apramycin, Colistin, Oxytetracycline and Lincomycin - Spectinomycin. Our data supports other findings of high antimicrobial resistance for Tetracyclines and Penicillins in samples from Portuguese slaughters. The antibioresistance for these antimicrobials groups has also been reported in swine samples in other European countries. This study provides information that together with data from other Portuguese studies, potentiate the knowledge of the real state of antimicrobials resistance in swine production and the adjustment of the antimicrobials administration.

Introduction

Antimicrobial resistance in bacteria from food animals is a worldwide public health issue. Enteric pathology is one of the main causes of economic losses in swine production. Resulting from viral or bacterial infection, enteric disease depends on several factors, as animals’ age, feed, biosafety conditions, among others. Prevention and treatment of enteric disease, as well as other swine diseases, lead antimicrobial therapeutics to make part of swine production systems.

Antimicrobial resistance in bacteria from food animals is a worldwide public health issue as it has been pointed out for the main organizations from Human and Animal Health, WHO, OIE, FAO (FAO, 2013; OIE, 2014; WHO, 2014). Antibiotics use in food animals can contribute to the emergence of antimicrobial resistance in bacteria that may be transferred to humans (Vieira, 2011). In Portugal, since 2014, the National Plan for Reduction of Antimicrobial use in Animals (DGAV, 2014), follows the European Commission Guidelines to promote human and animal health protection.

Diarrhoea associated with Escherichia coli can occur in young piglets and is a common cause of mortality and morbidity in post-weaning animals. The need of immediate therapeutic intervention, sometimes do
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One health approach under a concept of farm to fork

not allow sample collection for a previous antimicrobial sensitivity test. Therefore the Veterinary Diagnostic Laboratory has an essential role giving the Veterinary the information about data analysis and incidence of antimicrobial resistance of bacteria in different regions.

The aim of the present study was to collect data on antimicrobial resistance of Escherichia coli strains isolated in our laboratory, in samples collected from pigs with enteric disease symptoms, in Portuguese farms.

Material and Methods
A total of seventy seven E. coli isolates was collected from 26 swine farms, from the Center and South of Portugal, in 2013 and 2014. All the isolates originated from diarrhoea stools or swabs. Each sample was streaked onto MacConkey Agar plate followed by biochemical confirmation or Chromogenic CPS Agar plates and E. coli selection.

The susceptibility of E. coli isolates to a panel of 15 antimicrobials was tested using the disc diffusion method following MIC (Minimal Inhibitory Concentration) from Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2011). The following antimicrobial agents were tested: Amoxycillin (10 µg), Apramycin (15 µg), Ampicillin (10 µg), Cefotiofur (30 µg), Colistin (30 µg), Doxycline (30 µg), Enrofloxacin (5 µg), Flumequine (30 µg), Gentamicin (10 µg), Lincomycin (10 µg), Marbofloxacin (5 µg), Oxytetracycline (30 µg), Neomycin (30 µg), Lincomycin-Spectinomycin (109 µg) and Trimethoprim-Sulphamethoxazole (1.25 µg / 23.75 µg).

The resistance rates resulting from antimicrobial susceptibility test for the E. coli isolates from 2013 were compared to the ones of 2014, using the X2 test, considering statistically significant for p<0,05.

Results
The resistance rates of E. coli isolated from 2013 and 2014 to individual antimicrobials are shown in Figure 1. The 77 E. coli strains resulted resistant to at least one antibiotic. Most of the antibiotics tested found resistance in more than 50% of the E. coli isolates. All the strains were resistant to Lincomycin, though the resistance to Lincomycin-Spectinomycin was observed in 82% of the strains. Doxycline and Amoxycillin were also antibiotics with high resistance rates, 94% and 82%. On the other hand, Marbofloxacin (32%), Neomycin (13%) Cefotiofur (37%) and Gentamicin (30%) were the antibiotics with lower resistance rates in the E. coli isolates from this study. Antimicrobials resistance rates for the E. coli isolates from 2013 and 2014, frequencies comparing and X2 test results are presented in Table 1.

Discussion
Escherichia coli is usually a commensal bacterium of pigs, presenting a great potential for reserve and disseminate antimicrobial resistance. Thus E. coli is a good indicator to study antimicrobial resistance of bacteria found in animals, particularly in pigs, in the scope of our study.

In Portugal, several studies report antimicrobial resistance in pigs at slaughter, with high rates of resistance in E. coli isolates for Tetracyclines, Trimethoprim/sulphamethoxazole, Amoxycillin and Ampicillin. In this study, we also observed high rates of resistance for Doxycline (94%), Amoxycillin (88%), Ampicillin (82%) and Trimethoprim/Sulphamethoxazole (78%). Our data also show a significant increase of resistance rate, from 2013 to 2014, for Amoxycillin, Oxytetracyclin, as well as Apramycin, Colistin and Lincomycin-Spectinomycin. However, considering the small number of 2013 isolates, more isolates will allow to accurately infer about the tendency of these antimicrobial resistances.

In 2004, Pena and co-workers reported 100% of resistance to Amoxycillin, Oxytetracyclin and Sulphametoxazole from E. coli isolated from faeces of pigs at slaughter, in the Center of Portugal (Pena et al., 2004).

In 2008, the ARBAO-II (Antibiotic resistance in bacteria of animal origin – II) reported the results of a program for the continuous monitoring of antimicrobial susceptibility of pathogenic and indicator bacteria from food animals, with data from 17642 isolates of pathogens and indicator bacteria from fifteen European countries (Hendrickse, 2008). Data collected from Portugal, indicate a great resistance to Tetracyclin in E. coli isolates (98%) and Amoxycillin-Clavulanic acid, Ciprofloxacin and Gentamicin also found great resistance in Portuguese isolates comparing to other countries. The ARBAO-II study called the attention for the differences existing among several countries, in terms of antimicrobials availability, therapeutic procedures or biosafety measures and suggests that antibiotic choice should consider local pattern of antimicrobial resistance. In contrast with Portuguese data from ARBAO, in our study we found lower rate of resistance for Gentamicine.

Another Portuguese study from 2013, also report high resistance to Tetracyclin (93,9%), Trimethoprim/ Sulphametoxazole (69,7%) and Ampicillin (68,2%) in E. coli isolates from pig faeces, at slaughter (Ramos, 2013).

Costa e Machado (2014) reported 100% resistance to Spectinomycin and 95% to Neomycin, in 155 E. coli isolates from samples collected in animals and environment (water, air, feed) at 2 pig farms (Costa, 2014). These data differ from the lower rates of resistance to Neomycin found in our study, however the isolates
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Table 1 – Antimicrobial resistance rates for the E. coli isolates from 2013 and 2014 and p-value for the $X^2$ test

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>2013 Rate (n=61)</th>
<th>2014 Rate (n=16)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin</td>
<td>57%</td>
<td>53%</td>
<td>0.02150</td>
</tr>
<tr>
<td>Apramycin</td>
<td>57%</td>
<td>57%</td>
<td>0.03396</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>57%</td>
<td>57%</td>
<td>0.06236</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>27%</td>
<td>48%</td>
<td>0.04792</td>
</tr>
<tr>
<td>Cotrimoxazone</td>
<td>36%</td>
<td>16%</td>
<td>0.83940</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>44%</td>
<td>18%</td>
<td>0.06582</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>58%</td>
<td>32%</td>
<td>0.00900</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>24%</td>
<td>24%</td>
<td>0.75570</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>58%</td>
<td>18%</td>
<td>0.43280</td>
</tr>
<tr>
<td>Neomycin</td>
<td>32%</td>
<td>14%</td>
<td>0.4844</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>58%</td>
<td>14%</td>
<td>0.2457</td>
</tr>
<tr>
<td>Sulphamethoxazole</td>
<td>32%</td>
<td>14%</td>
<td>0.6844</td>
</tr>
</tbody>
</table>
from Costa’s study include environmental samples with the income of other resistances beyond the most commonly observed at slaughter, giving evidence on environmental dissemination of resistant bacteria.

High resistance to Tetracyclines, Amoxyclillin and Trimethoprim/Sulphamethoxazole has also been reported by other European countries as Spain (Teshager, 2000) or the United Kingdom (Enne, 2008).

The great resistance to Lincomycin observed in our study, was found by others that explain the fact by the common use of this antibiotic in pig enteric disease treatment (Hart, 2004).

Most of the Portuguese studies on antimicrobial resistance in pigs are carried out with samples from slaughter animals, having information on the region of origin. In contrast, samples from laboratory commonly miss information on animals’ region of origin or antimicrobials use on the collected samples. Animals age also influence the commensal flora of pigs (Bok, 2013) and it would be of great interest the antimicrobial resistance evaluation between different groups of ages. The reason to not compare groups of animals with different ages in this study was because age information was available for only 50% of the samples included in our data analysis.

In Portugal, the Tetracyclines group are the antimicrobials group with the highest sales, 36.5% from sales of antibiotics for animal use, followed by Penicillin and Macrolides (EMA, 2013). High consumption of these antibiotics is commonly pointed out as a reason for the antimicrobial resistance observed in food animals. The analysis of antibiotics use and correlation with antimicrobials resistance, over the time, would provide interesting information on time influence and cause-effect relations, important for the antimicrobial therapeutics plan to be applied by the Veterinarian in each farm.

The last report from ECDC (European Center for Disease Prevention and Control), about The European Antimicrobial Resistance Surveillance Network (EARS-Net), says that the main cause for the antimicrobial resistance in indicator bacteria are, probably, the selective pressure of antibiotics use in different food animals population. The report also points that the highest rates of resistance in E. coli isolates from pigs are for Ampicillin, Sulphamides and Tetracyclines, commonly used in animals (EFSA & ECDC, 2014). Nonetheless, EFSA, ECDC and several authors alert for the need of harmonized surveillance programs for continuous antimicrobials resistance monitoring, that allow accurate comparing among the different studies from several countries (Burow, 2014; Chantziaras, 2013; EFSA & ECDC, 2014)

Conclusion

This work was carried out in order to contribute for the knowledge of antimicrobial resistances in pigs, in Portugal. Data analyzed in this study demonstrate high rates of antimicrobials resistance in E. coli isolates from Portuguese pigs.

References

- EMA. 2013. Sales of Veterinary antimicrobial agents in 26 EU/EEA countries in 2012. Fourth ESVAC report. EMA-Veterinary Medicines Division
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