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# ENTEROHEMORRHAGIC *Escherichia coli* AND ANTIMICROBIAL RESISTANCE OF SWINES BRED IN THE STATE OF PARÁ

(*Escherichia coli* enterohemorrágica e perfil de resistência a antimicrobianos em suínos criados no estado do Pará)

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**ABSTRACT** - The aim of this study was to investigate an occurrence and antimicrobial resistance profile of enterohemorrhagic *Escherichia coli* in swine bred in Pará State. 200 swine fecal swine samples were collected and seeded in *Escherichia coli* and Gram negative broths, incubated and then on MacConkey Agar. Suspected colonies were identified on triple sugar iron agar for biochemical characterization and analysed from PCR Multiplex, followed by investigation of susceptibility to available antimicrobial agents using the system VITEK 2 *Compact (bioMérieux)™*. Of the 15 properties studied, six of them were verified by enterohemorrhagic *E. coli* (EHEC), being isolated in 11% (22/200) of the researched animals. The antimicrobial susceptibility test showed that 50% of the cases presented resistance to at least one antimicrobial: 31.81% for Nalidixic acid, 27.27% for trimethoprim-sulfamethoxazole, 18.18% for ampicillin and 13.63% for cephalothin. In this study, strains of enterohemorrhagic *Escherichia coli* were identified in swines and was possible to verify the resistance of these pathogenic strains to some antimicrobials used in veterinary and human routine, such as trimethoprim-sulfamethoxazole and ampicillin, which are indicated to combat them, emerging concern regarding the treatment of diseases involving pathogenic *E. coli*, as well as the selection of resistance genes in bacteria present in the animal microbiota used as a food, and the possibility of transferring these genes to bacteria of the human intestinal tract.

**Key words:** diarrhea; enteropathogens; swine farming.

**RESUMO -** O objetivo deste estudo foi investigar a ocorrência e o perfil de resistência antimicrobiana de *Escherichia coli* enterohemorrágica em suínos criados no Estado do Pará. 200 amostras fecais de suínos foram coletadas e semeadas em caldos *Escherichia coli* e Gram negativo, e posteriormente incubadas em Agar MacConkey. Colônias suspeitas foram identificadas em ágar tríplice açúcar ferro para caracterização bioquímica e analisadas a partir de PCR Multiplex, seguido de investigação de susceptibilidade aos agentes antimicrobianos disponíveis usando o sistema VITEK 2 *Compact (bioMérieux)®*. Das 15 propriedades estudadas, foram verificadas *E. coli* enterohemorrágica (EHEC) em seis, sendo isoladas em 11% (22/200) dos animais pesquisados. O teste de susceptibilidade aos antimicrobianos demonstrou que 50% dos casos apresentaram resistência a pelo menos um antimicrobiano: 31,81% para ácido nalidíxico, 27,27% para trimetoprim-sulfametoxazol, 18,18% para ampicilina e 13,63% para cefalotina. Neste estudo, cepas de *Escherichia coli* enterohemorrágica foram identificadas em suínos, e foi possível verificar a resistência dessas cepas patogênicas a alguns antimicrobianos utilizados na rotina veterinária e humana, como trimetoprim-sulfametoxazol e ampicilina, que são indicados para combatê-las, surgindo preocupação quanto ao tratamento de

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doenças que envolvem *E. coli* patogênica, bem como a seleção de genes de resistência em bactérias presentes na microbiota animal utilizada como alimento, e a possibilidade de transferência desses genes para bactérias do trato intestinal humano.

**Palavras-chave** - diarreia; enteropatógenos; suinocultura.

## INTRODUCTION

Enteric bacterial infections are an important disease in swine responsible for a huge impact on the swine industry worldwide, causing significant economic losses (Luppi, 2017). In addition, some of these pathogens can potentially be zoonotic, representing a risk to a collective health (Tseng et al., 2014).

These agents include enterohemorrhagic *Escherichia coli* (EHEC), also known as Shiga toxin-producing *E. coli* (STEC) or verotoxigenic toxin (VTEC). This pathogen is considered important because it causes causes diarrheal disease and the potentially lethal hemolytic uremic syndrome (Warr et al., 2021). In swines, STEC strains are known to be associated with edema disease, an often-fatal enterotoxemia in weaner and feeder swines, with major importance in porcine production, management and health (Tabaran e Tabaran, 2019).

Although *E. coli* infections are an old and well-known problem in swine farming, they remain a challenge for health professionals, as the intensification of animal production and greater reliance on antimicrobials to control infectious diseases and promote animal growth has contributing to the selection of resistant bacteria increasingly limit treatment options (Pissetti et al., 2017).

Regarding the Amazonian ecosystem, little is known about the occurrence of *E. coli* in swine and its resistance to antimicrobials. Thus, the aim of this study was to report the occurrence of enterohemorrhagic *E. coli* in swine raised in the State of Pará, diagnosed by PCR, besides verify the antimicrobial resistance profile of pathogenic strains in order to provide data that can be used to directly improve control measures.

## MATERIAL AND METHODS

Individual swab samples were collected and stored in a Cary Blair transport medium from 200 pigs from 15 farms located in the Mesoregions: Belém, Bujaru, Castanhal, Moju, Paragominas and São Miguel do Capim, with no predilection for race, age or sex, with or without diarrhea.

The samples were processed at the Enterobacterial Laboratory located at Evandro Chagas Institute (Ananindeua, Pará), where the fecal specimens were seeded in Escherichia coli (Difco, USA) and Gram negative (GN) broths; incubated at 35°C for 24

hours and then seeded on MacConkey - MC Agar (Difco, USA). Colonies with positive lactose were submitted to the Triple Sugar Iron Agar (TSI) screening media (Difco, USA). Biochemical characterization of bacterial isolates was performed using the VITEK 2 kit (bioMérieux®, Brazil).

The *E. coli* isolates were cultivated on Nutrient Agar (Difco, USA), and then the DNA was extracted according to the recommendations, through PCR amplification (Rocha et al., 2017) for subsequent amplification using the Multiplex PCR technique (Oligonucleotides and their respective amplification products in board 1).

**Board 1** - Oligonucleotides used in multiplex PCR and their respective amplification products.

| Designation | Sequence of oligonucleotides (5´- 3´) | target gene | Amplification product | Reference |
|---|---|---|---|---|
| eae-1<br>eae-2 | CTGAACGGCGATTACGCGAA<br>CGAGACGATACGATCCAG | *eae* | 917 pb | Aranda *et al.* (2004) |
| BFP-1<br>BFP-2 | AATGGTGCTTGCGCTTGCTGC<br>GCCGCTTTATCCAACCTGGTA | *bfpA* | 326 pb | Aranda *et al.* (2004) |
| aggRks-1<br>aggRksa-2 | GTATACACAAAAGAAGGAAGC<br>ACAGAATCGTCAGCATCAGC | *aggR* | 254 pb | Toma *et al.* (2003) |
| LT-f<br>LT-r | GGCGACAGATTATACCGTGC<br>CGGTCTCTATATTCCCTGTT | *let* | 450 pb | Aranda *et al.* (2004) |
| ST-f<br>ST-r | ATTTTTMTTTCTGATTTRTCTT<br>CACCCGGTACARGCAGGATT | *est* | 190 pb | Aranda *et al.* (2004) |
| IpaH-1<br>IpaH-2 | GTTCCTTGACCGCCTTTCCGATACCGTC<br>GCCGGTCAGCCACCCTCTGAGAGTAC | *ipaH* | 600 pb | Aranda *et al.* (2004) |
| VTcom-u<br>VTcom-d | GAGCGAAATAATTTATATGTG<br>TGATGATGGCAATTCAGTAT | *stx*1/<br>*stx*2 | 518 pb | Toma *et al.* (2003) |

PCR reaction was performed from 2 µL of each extracted DNA and 23 µL of the mix solution, containing between 0.5 to 1.5 µL according to each primer (Invitrogen, Brazil), 10 mM of dNTP mix dATP, dCTP, dGTP, dTTP (Invitrogen, Brazil Invitrogen, USA), 0.5 U of Taq DNA polymerase platinum, 1X Taq buffer, 50 mM MgCl2 (Invitrogen, Brazil) and ultra-pure sterile water for a final volume of 25 µL. The multiplex PCR preparations were placed in an automatic gradient thermocycler model Veriti ™ 96-Well Thermal Cycler (Applied Biosystems - USA) and subjected to specific amplification cycles that consisted of 1 step of 2 min at 50°C (Hot-Start), 1 step of 5 min. at 95 °C (Initial Denaturation) followed by 40 cycles of 1 s at 95 °C, 50 °C and 72 °C and 1 final 7 min extension step at 72 °C.

Amplicons were formed by electrophoresis on a 2% agarose gel, stained with ethidium bromide (10 mg / mL) in TBE buffer (0.89M Tris base, 0.45M Boric Acid, 1 mM

EDTA, pH 8.4) and visualized under UV light with the aid of a transluminator (Vilber Lourmat, France). As molecular size marker used the 1 Kb plus Ladder - Invitrogen, Brazil. Subsequently, the gel was photographed by a photodocumentation system, Biomaging Systems (UPV, USA).

Susceptibility to antimicrobial agents of isolates was evaluated following the recommendations of the Clinical and Laboratory Standards Institute - CLSI (2019), using the automated VITEK® 2 Compact (bioMérieux) card system for the antimicrobials Ampicillin, Amoxicillin, Piperacillin-Tazobactam, Cefalotin, Cefuroxime, Cefuroxime Axetil, Ceftriaxone, Cefepime, Ertapenem, Meropenemicin, Amicillin Nalidixic Acid, Ciprofloxacin, Norfloxacin, Nitrofurantoin, Trimethoprim- Sulfamethoxazole, in addition to the identification of enterobacteria producing extended-spectrum beta-lactamase (ESBL).

**RESULTS AND DISCUSSION**

Of the 15 properties studied, enterohemorrhagic (EHEC) was found in six, being isolated in 11% (22/200) of the animals. Of the 22 animals in which EHEC was isolated, 10 were male and 12 female; and only five animals were older than one year, with the remaining positive (17) has less than four months. The prevalence of this organism in this study was higher than in previous studies (Franco et al., 2010; Machado et al., 2014) and lower than others (Meng et al., 2014). This can be justified by the variation of the sample type and the methodology used.

Regarding the clinical symptomatology of EHEC in positive animals, the diarrheal condition was present in two properties. Other symptoms were third eyelid edema, and neurological symptoms (characterized by motor incoordination, paresis, imbalance, pedalling movements, and seizures, followed by death) in two other properties.

In acute cases of edema disease caused by enterohemorrhagic *E. coli*, subcutaneous edema occurs, mainly the eyelids, ears, forehead, nose and lips, besides a history of diarrhea with blood streaks (Santos e Alessi, 2010); this was observed in animals of the present study and confirmed the presence of EHEC in feces by PCR.

As reported the verified neurological signs may occur by focal symmetric encephalomalacia, or porcine cerebral angiopathy; morbidity being approximately 35% and mortality 100% (Cheng et al., 2006; Mcgavin e Zaachary, 2013).

Importantly, the EHEC was isolated from the feces of seven and 15 animals with and without clinical signs, respectively. The production of one or more Shiga toxins, isolated, is not sufficient to cause the disease (Remfry et al., 2021) which may justify the

fact that some animals show no clinical sign; in addition, other intrinsic and extrinsic factors may have been determinant, such as age, immune status, nutritional and genetic constitution of animals, passive transfer of specific antibodies by colostrum, environmental contamination, and stress factors such as extreme temperatures, overcrowding and intercurrent infections by other agents, besides the presence of plasmid pO157, which encodes enterohemolysin and the production of fimbrial and afimbria adhesions (Santos e Alessi, 2010; Mcgavin e Zaachary, 2013).

The prevalence of EHEC in clinically healthy swine populations has been reported and the prevalence of EHEC, being higher when diagnosed by PCR (25.42%) (Feder et al., 2003; Cornick e Helgerson, 2004; Meng et al., 2014). Isolated EHECs in clinically healthy pigs may represent a potential risk to humans, although other authors consider this a low potential, based on comparison of serotypes and presence of virulence genes in porcine and human strains (Zweifel et al., 2006; Meng et al., 2014).

In the investigated properties, the animals were confined in the same bay and *E. coli* can be transmitted horizontally between pigs housed in confinement conditions (Menin et al., 2008). Under these conditions, the transmission between pigs of this microorganism occurs through contaminated aerosols (Cornick e Vukhac, 2008). None of the properties in which EHEC was isolated had direct contact between swines and cattle, indicating that the infection of the bacteria remains among the pig population (Menin et al., 2008). Even in some studies, surprisingly, the prevalence in swine samples (10.8%) was higher than in cattle (2.9%), suggesting that pigs may be an important source of this microorganism (Ateba e Mbewe, 2011).

Regarding antimicrobial susceptibility search, of the 22 samples tested, 50% of the isolates were resistant to at least one antimicrobial agent: being 31.81% to nalidixic acid, 27.27% to trimethoprim-sulfamethoxazole, 18.18% to Ampicillin and 13.63% for Cephalothin. No ESBL-producing isolates were detected.

This result was expected, since *E. coli* is one of the main species whose plasmids contain genes involved in the process of multiple antimicrobial resistance, besides having a short interval between generations and mechanisms of transfer of genetic material, being this a feature related to its large environmental distribution (Sherley et al., 2004). In addition, antimicrobial multi-resistant E. coli strains are common on pig carcasses and present highly diverse genotypes and resistance phenotypes and genotypes (Pisseti et al., 2017).

Multidrug resistance was checked in *E. coli* samples isolated from steers in Concórdia, SC region, with the highest resistance to tetracycline (82.3%), nalidixic acid

(64%), ampicillin (41%), and trimethoprim-sulfamethoxazole (36%); similar to the present research (Silva et al., 2008).

In evaluating the antimicrobial resistance of strains of *E. coli* isolated from swine samples from the States of Rio de Janeiro, Minas Gerais, Paraná and Santa Catarina, found at least seven resistance to antibiotics (carbenicillin, ceftazidime, clindamycin, chloramphenicol, erythromycin, penicillin and rifampicin) routinely used in the treatment of foodborne diseases of all strains of pathogenic *E. coli*, being sensitive only to gentamicin and tobramycin (Franco et al., 2010).

In the analysis of samples from 15 pig farms located in the States of Rio Grande do Sul, Santa Catarina, Paraná, Minas Gerais and Goiás; the antimicrobial susceptibility of the isolates presented resistance levels above 60% for amoxicillin, ampicillin, ciprofloxacin, enrofloxacin, streptomycin and trimethoprim-sulfamethoxazole; being the highest indices (96.3%) observed for tetracycline (Sato et al., 2015).

Nalidixic acid and trimethoprim-sulfamethoxazole were also the highest resistances found when analyzing enterohemorrhagic *E. coli* isolated from pigs in China (Meng et al., 2014).

The difference in results between the mentioned researchers and the present study was expected, since the resistance profile of these bacteria to antimicrobials has a huge variation due to the strict relationship with the history of antimicrobial use in each population and region.

The uncontrolled use of antibiotics in production animals is worrying not only from the perspective of the veterinary clinic, but also from a zoonotic aspect as well, as it may contribute to the prevalence of resistance in human *E. coli* strains (Marshall e Levy, 2011).

## CONCLUSIONS

Enterohemorrhagic *E. coli* were identified in pigs, highlighting the resistance of these pathogenic strains to some antimicrobials used in veterinary and human routine, which are indicated to combat them.

There is concern about the treatment of diseases involving pathogenic E. coli, as well as the selection of resistance genes in bacteria present in the animal microbiota used as a food, and the possibility of transferring these genes to bacteria of the human intestinal tract.

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**INFORMATION NOTE**

This project was approved by the Ethics Committee (CEUA) of the Federal Rural University of Amazonia - UFRA, Protocol 038/2013, and process 23084.010923 / 2013-15.

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