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Viability of enterobacteria in swine manure storage units

Abstract – The objective of this work was to evaluate the efficiency of the stabilization process of swine manure in storage ponds regarding the presence and antibiotic resistance of pathogenic bacteria. The study was carried out in two trials in ten swine farms located in the municipality of Presidente Castello Branco, in the state of Santa Catarina, Brazil. In the first trial, the efficiency in reducing *Escherichia coli* (E_C), non-*E. coli* coliforms, and *Salmonella* sp., as well as the resistance of isolates to antimicrobials, was investigated under recommended handling conditions. Sampling was done at three depths and two times. In the second trial, the concentration of total and fecal coliforms (E_C) was evaluated under field handling conditions, when the manure was distributed on the field. Sampling depth does not influence the concentration of E_C and *Salmonella* sp., and isolated bacteria are multi resistant to the evaluated antibiotics. Hydraulic retention time is determinant in reducing the population levels of the indicator agents. The obtained results are indicative that swine manure handling must be carried out according to sanitary standards, in order to minimize the propagation risk and antimicrobial resistance of pathogenic microorganisms.

Index terms: *Escherichia coli*, *Salmonella* sp., one health, swine farming.

Viabilidade de enterobactérias em unidades de armazenamento de dejetos líquidos de suínos

Resumo – O objetivo deste trabalho foi avaliar a eficiência do processo de estabilização de dejetos suínos em lagoas de estabilização quanto à presença e à resistência a antibióticos de bactérias patogênicas. O estudo foi realizado em dois ensaios, em dez granjas de suínos localizadas no município de Presidente Castello Branco, no estado de Santa Catarina, Brasil. No primeiro ensaio, investigou-se a eficiência na redução de *Escherichia coli* (E_C), coliformes não *E. coli* e *Salmonella* sp., bem como a resistência de isolados a antimicrobianos sob condições de manejo recomendadas. A amostragem foi realizada em três níveis de profundidade e dois tempos. No segundo ensaio, avaliou-se a concentração de coliformes totais e fecais (E_C) em condições de manejo a campo, no momento da distribuição do dejeto na lavoura. A profundidade de amostragem não influencia a concentração de E_C e *Salmonella* sp., e as bactérias isoladas são multirresistentes aos antibióticos avaliados. O tempo de retenção hidráulica é determinante na redução dos níveis populacionais dos agentes indicadores. Os resultados obtidos são indicativos de que o manejo dos dejetos suínos deve ser realizado de acordo com as normas sanitárias, para minimizar o risco de propagação e a resistência antimicrobiana de microrganismos patogênicos.

Termos para indexação: *Escherichia coli*, *Salmonella* sp., saúde única, suinocultura.

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Introduction

The application and distribution of manure on croplands is influenced by the relief and geographical position of farms and the size of the area, being limited to certain times of the year depending on weather conditions (Hodgson et al., 2016; Broetto & Meinerz, 2017). Therefore, until its disposal, manure needs to be kept in a suitable storage structure and to be correctly managed, which requires understanding the basic types of bacteria present in it. According to Chastain & Henry (2015), three classes of bacteria capable of breaking down organic matter are found in animal waste: aerobic, anaerobic, and facultative.

One of the main problems related to intensive animal production is, therefore, the contamination of the environment by pathogenic microorganisms from manure, associated with their resistance to antimicrobial agents (Silva et al., 2015). The concentration of fecal indicator organisms, such as *Escherichia coli* and intestinal enterococci, in manure or wastewater, however, varies greatly, depending on manure type and storage management, as well as on the environmental conditions of the area (Hodgson et al., 2016).

In this context, the anaerobic treatment of liquid or semiliquid manure is important because it is responsible for the stabilization and reduction of pathogenic organisms that, after contaminating the environment, may be hazardous to human and animal health (García-González et al., 2016). For that treatment, the storage of manure in a pond system, for example, is effective and inexpensive for swine producers, especially considering the saturation of land areas available for spreading the manure (Watabe et al., 2003), as observed in Southern Brazil, where large amounts of swine manure are disposed in small units of land area.

For swine production in that Brazilian region, the environmental institute of the state of Santa Catarina – Instituto do Meio Ambiente (IMA) – established criteria in Instrução Normativa 11 – Suinocultura (Fatma, 2014). In this first version of the document, it is recommended that manure (digestate) be subjected to fermentation or to a minimum hydraulic retention time (HRT) of 60 days before being applied to pastures. However, in the most recent version, there is no mention of a minimum number of days for fermentation/retention, but of the use of digestate from only one pond with either total storage capacity or a HRT of no less than 40 days (IMA, 2021).

These criteria are essential for the reduction of enteropathogenic microorganisms in animal manure, aiming to minimize the risk of diseases caused by the contamination during production of vegetables and fruits destined for human consumption in natura (Venglovsky et al., 2018). Therefore, the policies for the use and consumption of a certain product after the application of waste must be carefully followed (Ashekuzzaman et al., 2018).

Regarding the microbiological quality of reused wastewater, the used standards are not consistent worldwide. In Brazil, for example, there are no established guidelines, whereas, in the United States, each state defines its own quality standards. In Florida and Texas, for instance, the maximum allowable value for fecal coliforms is $8x10^2$ colony forming units (CFU) 100 mL^{-1} (Jeong et al., 2016).

Among the microorganisms affecting human, animal, and environmental health, *Salmonella* sp. and *Escherichia coli* (E_C) stand out (Bilotta & Kunz, 2013; Betancur H. et al., 2016). Specifically in commercial swine production, the control of E_C is a challenge due to its recurring antimicrobial resistance, which makes the therapeutic control of the caused diseases difficult (Silva et al., 2015; Venglovsky et al., 2018).

In animal production, antibiotics are normally used for therapeutic purposes in the prevention and cure of diseases and for nutritional therapies (Noschang et al., 2017). However, due to antimicrobial resistance (Santos et al., 2016; Noschang et al., 2017; Costa et al., 2019), there is a tendency towards the controlled use of those substances, whose residues may cause damage to the homeostasis of one health as a result of the selection of resistant strains (Santos et al., 2016).

The objective of this work was to evaluate the efficiency of the stabilization process of swine manure in storage ponds regarding the presence and antibiotic resistance of pathogenic bacteria.

Materials and Methods

To investigate the effect of storage time on the profile of the bacteriological indicators of swine manure during its stabilization process, two trials with different experimental handling conditions were carried out. In the first and second trials, respectively, HRT according to Instrução Normativa

11 – Suinocultura (Fatma, 2014) and the operational HRT or field handling were adopted.

The experiment was performed in the largest swine-producing region of Santa Catarina, Brazil, located in the municipality of Presidente Castello Branco, in the socioeconomic micro-region of Alto Uruguai Catarinense, belonging to the Jacutinga River watershed, which is positioned among the municipalities of the western mesoregion of the state (27º17'00"S, 51º48'00"W, at 649 m of altitude). The area was chosen due to its high concentration of 42.2 pigs per inhabitant (IBGE, 2020; Santa Catarina, 2020).

Ten properties (farms), named A to J, representing 10.87% of the total number of swine farms in the municipality, were selected for the study according to the following criteria: presence of an agro-industrial integration system, similar rearing system (finishing), controlled feed management, and manure storage in covered ponds.

In the first trial, two samplings were carried out to evaluate the recommended handling conditions in relation to storage time. In the first (T_0), samples were collected directly from the covered storage units (ponds), regardless of the volume of stored manure. From T_0, in the second trial (T_{60}), the entire input and output flow of waste was interrupted for a period of 60 days. The used HRT was based on the minimum recommended by Fatma (2014).

To evaluate the effect of depth on the population of bacteriological indicators, three samples of 100 mL were collected from each manure pond at the following three depths: 0–10, 10–20, and 20–30 cm. The samples were collected using a previously sterilized 300 mL stainless steel rod sampler and then transferred to sterile bottles, which were placed in a previously cooled expanded polystyrene box and immediately transported to the reference laboratory for microbiological analyses.

The analyses for enterobacteria – E_C and non-E_C coliforms (E_{NEC}) – were performed according to Sant'ana et al. (2003) with adaptations. The samples collected at the three depths were subjected to dilutions (base 10) in 0.1% peptone water (Merck KGAA, Darmstadt, Germany), and 1.0 mL of the dilutions was seeded in Chromocult Coliform Agar (Merck KGAA, Darmstadt, Germany) using the pour plate technique. The plates were incubated at 37°C for 24 hours, and the colonies were then counted. Violet colonies were classified as E_C and pink colonies, as non-E_C coliforms. Total coliforms correspond to the total count of the violet and pink colonies.

The qualitative analysis of *Salmonella* sp. was carried out according to Michael et al. (2003). Samples from the three depths were diluted in peptone water (1:10) and incubated at 37°C for 24 hours (25 g for 225 mL). A 1.0 mL aliquot was sub-cultivated into 9.0 mL of the Müller-Kauffmann Tetrathionate broth (BD Difco, Franklin Lakes, NJ, USA), and a 0.1 mL aliquot, into 9.9 mL of the Rappaport-Vassiliadis selective enrichment broth (Merck KGAA, Darmstadt, Germany); incubation was done at 42°C for 24 hours. Subsequently, aliquots of the selective broths were seeded on the selective plating medium Xylose-lysine-tergitol 4 (XLT4) agar (BD Difco, Franklin Lakes, NJ, USA) and on the 1.0 L Brilliant Green agar plus 1.0 mL of 4.0% novobiocin (BD Difco, Franklin Lakes, NJ, USA). After 24 and 48 hours of incubation, the colonies suspected to be *Salmonella* sp. were isolated and subjected to biochemical tests to confirm their identification.

The *Salmonella* sp. colonies obtained in the qualitative test were used for the quantitative analysis, being enriched in the Modified Semi-Solid Rappaport-Vassiliadis (Merck KGAA, Darmstadt, Germany) medium with novobiocin. After the plates were incubated for 24–48 hours at 41.5°C, the medium was checked for color change, which was confirmed by seeding its contents on the selective XLT4 agar medium according to the ISO/TS 6579-2:2012 standard (ISO, 2012). The most probable number (MPN) was calculated using the MPN calculation program, version 5 (Jarvis et al., 2010).

To assess the antibiotic resistance profile, the disc-diffusion methodology was used, following Clinical and Laboratory Standards Institute (CLSI, 2017) with adaptations. For this analysis, three isolated strains from laboratory tests (two E_C and one *Salmonella* sp. from farms A and E) and two reference strains (*Salmonella enterica Typhimurium* – ATCC 14028 and *Escherichia coli* – ATCC 25922) from Fundação Oswaldo Cruz (Rio de Janeiro, Brazil) were used.

The bacterial inocula were standardized at 10^7 CFU mL^{-1} using a calibration curve calculated from data obtained from optical density measurements and the count of CFU. The inoculum mat was prepared in the

Mueller-Hinton Agar culture medium (Oxoid, Thermo Fischer Scientific Inc., Nepean, Canada) with a sterile swab. Thirteen types of antibiotics were tested: 10 U penicillin G, 10 µg gentamicin, 10 mcg norfloxacin, 25 µg sulfazotrim, 5 mcg ciprofloxacin, 30 mcg tetracycline, 30 µg chloramphenicol, 10 mcg ampicillin, 30 µg cephalothin, 30 µg cephalexin, 20 µg amoxicillin + 10 µg clavulanic acid, 15 µg erythromycin, and 10 µg streptomycin. The antibiotic discs were randomly distributed on the plates, and the assay was performed in triplicate for all samples. The plates were kept at 37ºC, for 18–24 hours, in the EL202/3 incubator of the BOD type (Eletrolab, São Paulo, SP, Brazil). When present, the inhibitory halos were measured using the 1108-300 digital caliper (INSIZE do Brasil, São Paulo, SP, Brazil) and classified according to CLSI (2017).

In the second trial, field handling was analyzed to determine the concentration of the microbiological indicators at distribution time on the land. For this, samples were obtained from nine manure ponds from the eight farms used in the first trial. For a greater representativeness of manure handling, the samples were collected at the time of disposal. The HRT of manure at distribution time was 15, 20, 35, and 120 days for two, three, two, and one sample replicates, respectively. Using a submerged pump, the manure from each pond was pumped until full truck capacity (11 or 12 m^3) and then transported to the disposal area, where sampling was done at distribution time to assure representativeness and homogeneity. After collected, the samples were properly stowed in previously cooled expanded polystyrene boxes and immediately transported to the reference laboratory for analysis.

For the analysis of enterobacteriaceae (total and fecal coliforms), sampling met the criteria required for the quantitative colorimetric analysis (Hall, 2017), which includes using the 9222 membrane filtration technique for the quantitative determination of total (C_T) and fecal (C_F in E_C) coliforms; the latter was expressed in E_C. Two 100 mL volumes of each sample (after 1:100 dilution) were filtered on filter membranes (∅=0.45 µm) that were aseptically placed in Petri dishes containing the m-Endo and m-FC culture media specific for C_T and C_F and were incubated in a bacteriological oven at 35 and 45°C, respectively, for 24 hours. The CFU were then quantified and expressed as CFU 100 mL^{-1}.

For the statistical analysis, zero occurrence and upper-limit values were assigned, respectively, to all samples below and above the detection limits. All counts of E_{NEC}, E_C, C_T, and C_F in E_C transformed into $\log_{10}$ (CFU + 1) were used for the analysis. To analyze the relationship between the studied variables, the data were evaluated by Pearson's correlation analysis in PROC CORR (SAS Institute Inc., Cary, NC, USA). The effect of depth and collection time on E_{NEC} and E_C was determined using mixed-effects models, and means were compared by Tukey's test. The effect of HRT on C_T and C_F was determined using a regression model. The p-value of <0.05 was considered the level of significance in all statistical tests. All statistical analyses were performed using the SAS, version 9.3, software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Considering the profile of E_{NEC}, E_C, and *Salmonella* sp. under recommended handling conditions, there was a strong negative correlation between the E_C indicator and collection time ($r = -0.82$, $p < 0.0001$) and a moderate negative correlation between E_{NEC} and collection time ($r = -0.54$, $p < 0.0001$) (Table 1), but lower by 28% compared with E_C. This shows that the E_C indicator is the most reliable for evaluating the effectiveness of the removal of indicator bacteria during the stabilization process of swine manure in storage units.

No interaction, however, was observed between depth and collection time for E_{NEC} and E_C enterobacteria counts ($p = 0.9058$ and $p = 0.9568$, respectively). Although depth did not affect E_{NEC} ($p = 0.8871$) and E_C ($p = 0.9817$), collection time affected both ($p < 0.0001$) (Table 1). Therefore, there was a reduction of 87.09 and 97.77%, respectively, in the concentration of E_{NEC} and E_C, in alignment with Venglovsky et al. (2018), who found a decrease of ≤ 3 $\log_{10}$ E_{NEC} and E_C after 55 days of retention at a controlled temperature of 42°C. Despite this result, these authors recommend that a HRT of 115 days be respected for the safe use of manure. Santos et al. (2007) reported a constant concentration of E_{NEC} and E_C during an observation period of 120 days, but emphasized that this was due to the continuous input of manure into the studied system. Betancur H. et al. (2016) highlighted the persistence of E_{NEC} and E_C after manure treatment in digesters, which may be related to the used treatment system and HRT.

In the literature on animal waste, Venglovsky et al. (2018) studied the risks of the application of slurry to

the land used for agricultural production or grazing, especially regarding the viability of *Salmonella typhimurium*, *Escherichia coli*, total coliforms, *Ascaris suum* eggs, and fecal enterococci at different storage temperatures and times. García-González et al. (2016) monitored E_C and *Salmonella* sp. indicators in eight swine waste treatment systems (six of them with anaerobic digestion) and observed initial pathogen concentrations of $1.7x10^5$, $5.6x10^5$, and $8.2x10^3$ CFU 100 mL^{-1} E_C in the anaerobic systems. All plants with anaerobic digestion were able to eliminate *Salmonella* sp. and reduce E_C below 3 log_{10}, which decreases the risk of pathogens contaminating the environment when the digested effluent is distributed on agricultural soil.

In the present study, *Salmonella* sp. was positively identified in farms A and B only in the post-storage sample; however, no growth was observed in the quantitative analysis. The presence of the microorganism in the sample from farm A is possibly related to the contamination of the manure by vectors from the environment. Santos et al. (2007) reported the occurrence of *Salmonella* sp. after the longest storage period when monitoring the presence of pathogenic indicators in manure samples stored for 0, 30, 60, 90, and 120 days after being collected at the opposite side of the pond inlet point in three swine farms. These authors concluded that the small presence of this pathogen shows that it is a very poor competitor, which means that, post-storage, it may be isolated in the laboratory after the decline of the multiplication rate or inactivation of other microorganisms.

In the sample from farm B, the presence of *Salmonella* sp. in the post-storage sample may have been due to the handling error of the farmer, who did not follow the HRT proposed in the present study. This shows that if the HRT is not respected, undesirable pathogens may develop in the material to be distributed on the soil.

Fortnightly during ten months, Schmidt & Cardoso (2003) also monitered the presence of *Salmonella* sp. in manure, which was treated in anaerobic, facultative, mechanically aerated, and aerobic ponds. The authors concluded that the unit with the lowest pathogen count – 20% (4/20) of the samples – was the anaerobic one.

Watabe et al. (2003) examined the prevalence of fecal pathogens in 43 samples collected from swine unseparated slurry (14), separated solids (16), and separated liquid (13) during 90 days, finding a significant reduction of microorganisms in the solid component after the mechanical separation process, which shows the need for proper management to reduce the transmission of pathogens to the environment.

In the disc-diffusion antimicrobial resistance tests, multidrug-resistant strains, i.e., unsusceptible to antimicrobial agents according to the parameters proposed by CLSI (2017), were found. Pornsukarom & Thakur (2016) and Brisola et al. (2019) added that, when bacterial strains are resistant to three or more drugs, they are considered multidrug resistant. The strains isolated from the evaluated manure samples, as well as the reference strains, were sensitive to gentamicin, but resistant to penicillin, tetracycline, and erythromycin, besides being moderately susceptible to the other drugs (Table 2). The resistance of all strains to tetracycline and erythromycin may be due to a common and inappropriate use of antibiotics in animal production in the studied farms, whereas the sensitivity of all strains to gentamicin suggests that this medicine is a possible choice for antibiotic therapy in these farms.

To determine the spread of antimicrobial-resistant *Salmonella* sp. in the environment due to the application of manure in commercial swine farms in two American states, Pornsukarom & Thakur (2016) carried out a study using 189 samples of the pathogen isolated from 1,430 manure samples in 21 days. The isolated samples showed 88.36, 67.2, and 57.67%

Table 1. Effect of depth and collection time on counts of non-*Escherichia coli* enterobacteriaceae (E_{NEC}) and *E. coli* coliforms (E_C) in a swine manure pond in the municipality of Presidente Castello Branco, in the state of Santa Catarina, Brazil[(1)].

Variable	Depth (cm)			Collection		r	Mean	Standard error	Pr>F		
	0–10	10–20	20–30	Pre	Post				D	C	P*C
E_{NEC}	1.99	1.86	2.07	2.83a	1.11b	-0.54	1.97	0.2078	0.8871	<0.0001	0.9058
E_C	1.99	1.92	1.93	3.46a	0.44b	-0.82	1.95	0.2403	0.9817	<0.0001	0.9568

[(1)]Pre, pre-storage; Post, post-storage; r, correlation coefficient; D, depth; C, collection; and Pr>F, probability. Different lower case letters, in the rows, differ by Tukey's test, at 5% probability. Numbers in bold are statistically significant at 5% probability.

resistance against streptomycin, sulfisoxazole, and tetracycline, respectively.

Watabe et al. (2003) found that *Salmonella* sp. had multidrug resistance to tetracycline (100%) and sensitivity to gentamicin in 29 colonies of the pathogen isolated from the collected samples. Oliveira et al. (2020) concluded that *Salmonella* sp. was 59% resistant to tetracycline and that all 63 analyzed samples were sensitive to gentamicin, as observed in the present study.

In relation to E_C, Pissetti et al. (2021) conducted a controlled study to verify the antimicrobial resistance of this microorganism in fecal samples from pigs divided, from birth to finishing (120 days), into four groups (n = 48), of which three received an antibiotic protocol and one did not. Of the samples collected by these authors, 302 containing E_C were isolated and then subjected to an antimicrobial resistance test, which showed a high pathogen resistance to nalidixic acid, sulfonamide, and tetracycline, emphasizing the need for a responsible use of antibiotics. Brisola et al. (2019), however, found that the investigated E_C strains were sensitive to gentamicin and resistant to sulfazotrim, as in the present study. According to these authors, these data are important for public health in Brazil, a large meat producer, where the antimicrobial resistance of pathogens of animal origin is still unknown. Moreover, these microorganisms can cause diseases in humans and animals that are even more difficult to treat when resistance genes spread between strains of the same or different species, showing the need to rationally use antimicrobials and properly treat animal waste.

Bacterial resistance can either occur naturally, following the introduction of a particular drug into the environment, or develop in an acquired way, through genetic mutations and competitive pressure from strains resistant to the used antimicrobial agent (Costa et al., 2019). The resistance genes present in each microorganism are located on the chromosome of the agent and in transmission plasmids (Laxminarayan et al., 2013); the latter are mobile genetic elements that have the capacity of transmitting genetic material horizontally between microorganisms, perpetuating genes that confer antimicrobial resistance (Brisola et al., 2019).

Furthermore, drug residues can cause damage to one health balance, which has been leading to a controlled use of antimicrobial substances during the production of animal protein (Santos et al., 2016). The use of some drugs in animal production has already been banned in Brazil, such as tylosin, lincomycin, and thiamulin (Brasil, 2020), as well as in several other countries (Noschang et al., 2017).

Under field handling, there was a strong negative correlation between C_T and HRT ($r = -0.87$, $p<0.0005$) and between E_C and HRT ($r = -0.86$, $p<0.0005$), indicating the prevalence of HRT in pathogen concentration and that retention time is essential for manure stabilization, reducing the risk to one health due to pathogenic bacteria deposited on the soil (Table 3).

Table 2. Susceptibility of pathogenic strains to antimicrobial agents in a swine manure pond in the municipality of Presidente Castello Branco, in the state of Santa Catarina, Brazil[(1)].

Active ingredient	Indicator bacteria (strain origin)				
	Escherichia coli (Farm B)	*E. coli* (Farm A)	*E. coli* (ATCC 25922)	*Salmonella* sp. (Farm A)	*Salmonella enterica* Typhimurium (ATCC 14028)
10 U penicillin G	R	R	R	R	R
10 µg gentamicin	S	S	S	S	S
10 mcg norfloxacin	S	R	S	I	S
25 µg sulfazotrim	S	R	R	S	S
5 mcg ciprofloxacin	I	R	S	R	S
30 mcg tetracycline	R	R	R	R	R
30 µg chloramphenicol	S	R	S	S	S
10 mcg ampicillin	R	R	S	R	S
30 µg cephalothin	R	R	I	I	R
30 µg cephalexin	R	I	R	S	S
Amoxicillin + 30 µg clavulanic acid	R	I	S	S	R
15 µg erythromycin	R	R	R	R	R
10 µg streptomycin	S	R	R	R	R

(1) R, resistant; S, sensitive; and I, intermediate.

Table 3. Effect of hydraulic retention time on total (C_T) and fecal (C_F) coliform counts in a swine manure pond in the municipality of Presidente Castello Branco, in the state of Santa Catarina, Brazil[(1)].

Variable (log_{10} CFU 100 mL^{-1}))	Hydraulic retention time (days)				r	Mean	Standard error	R^2	Pr>F			Regression equation
	15	20	35	120					L	Q	C	
C_T	6.91	6.83	5.50	3.70	-0.87	6.21	0.4022	0.76	0.0473	0.1384	0.5053	Y = 7.24168 - 0.03131x
C_F (E_C)	5.89	6.07	4.29	2.48	-0.86	5.22	0.4473	0.74	0.0415	0.1174	0.2030	Y = 6.34545 - 0.03440x

[(1)]CFU, colony-forming units; r, correlation coefficient; R^2, coefficient of determination; Pr>F, probability; L, linear effect; Q, quadratic effect; and C, cubic effect. Numbers in bold are statistically significant at 5% probability.

After collecting 37 manure samples from primary anaerobic ponds in sow, nursery, and finishing farms, McLaughlin et al. (2009) found mean concentrations of C_T and E_C in the order of 1.4×10^5 and 5.4×10^4 CFU 100 mL^{-1} (n = 10) in the stabilization ponds under the finishing system. Regarding the use of the population of these indicators in crop and pasture fertigation, there are still no regulatory standards in Brazil.

The average HRT of the manure observed under field conditions was 33 days. For exploratory purposes, if the sample with a HRT of 120 days – which was still stored due to fermentation problems – was excluded, the average would be 22 days, i.e., 50% less than the recommended by the Brazilian environmental agency (Fatma, 2014).

The average fecal coliform (E_C) concentration observed in the present study was $1.29x10^2$ CFU 100 mL^{-1} in the first trial and $4.1x10^5$ CFU 100 mL^{-1} in the second, reflecting the variation in stabilization time between the recommended handling and field conditions. Therefore, handling swine manure without following the current laws may cause the propagation of pathogenic microorganisms, which, associated with the antimicrobial resistance of bacteria, would negatively affect the homeostasis of one health. According to Venglovsky et al. (2018), the reduction of enteric pathogens in swine manure before its application to pasture or cropland is key to mitigate the risk of pathologies transmitted by microorganisms.

The obtained results are indicative that the responsible use of antimicrobial drugs according to the current legislation allows of a safe animal production, from the sanitary and environmental point of view, both indispensable for a sustainable industrial process. Therefore, the studied topic should receive special attention, especially in Brazil, an important producer of animal protein in the world scenario.

Conclusions

1. Hydraulic retention time (HRT) is determinant in reducing indicators of pathogenic microorganism populations as *Escherichia coli*, non-*E. coli* coliforms, and *Salmonella* sp., and the average HRT of the swine manure pond under field conditions is 33 days.

2. *Escherichia coli*, non-*E. coli* coliforms, and *Salmonella* sp. isolated from swine manure ponds are multi resistant to the evaluated antibiotics.

3. The depth of the manure ponds does not influence the concentration of enterobacteria.

4. Swine manure handling must be carried out in agreement with sanitary standards, in order to minimize the propagation risk of pathogenic microorganisms and their resistance to antimicrobial agents.

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