Prevalence and some Virulence Factors of *Salmonella* spp Isolated from Pigs and Piggery in Port Harcourt Metropolis.

Abstract

Piggery habitats are a substantial reservoir and are frequently asymptomatic carriers of the bacterium, *Salmonella*. *Salmonella* can be shed in faeces, and urine allowing infection to spread to other pigs and the environment. Therefore, there is a need to determine the prevalence and some virulence factors of *Salmonella* spp in pigs and piggery. The study areas were M & K pig farm Alakahia (station A) and Rivers State University pig farm (station B). A total evaluation of 112 samples ~~were~~ wasobtained with seven sample types comprising of faeces, floor, food trough, foreskin, urine, walls, and water troughs were aseptically collected. Samples were examined for the presence of *Salmonella* using the standard microbiological approach for enumeration and identification. Mean *Salmonella-Shigella* counts (SSC) feacal sample ranges for stations A and B were: 1.6 to $3.1x10^5$cfu/g, floor $5x10^4$ to $2.0x10^5$cfu/m^2, food trough $3x10^4$ to $1.2x10^5$cfu/m^2, Foreskin $4x10^4$ to $1.4x10^5$cfu/m^2, urine 0 to $1x10^4$cfu/ml, Wall $5x10^4$ to $2x10^5$cfu/m^2, Water trough $6x10^4$ to $1.3x10^5$cfu/m^2. Seventy-five isolates of *Salmonella* belonging to 5 species were isolated which includewith feacal sample recording the highest prevalence (10.7%) at both locations. The virulence test performed on ~~the~~ all *salmonella* isolates were 100% motile, haemolysis, catalase and S. *typhimurium* (80*%),* S. *choloraesius* (89.4%), S. *enterica* (93.3%)S.*enteritidis* (54.5%) and S. *bongori* (100%)to biofilm production test. The high prevalence and virulent factors observed in this study indicate a high potential risk of transmission of *salmonella* spp in piggery, which can have ~~a~~ serious implications~~to~~ forpublic health. ~~It is essential that a more effective control strategies~~A more effective control strategies must be employed to minimize the prevalence of *salmonella* spp in pigs and piggeryin the Port Harcourt metropolis.

Keywords: Prevalence, Virulence Factors, *Salmonella* spp, Pigs, Piggery.

Introduction

Pigs are a substantial reservoir and are frequently asymptomatic carriers of this pathogen. *Salmonella* can be deposited in the feces allowing infection to spread to other pigs, the environment, transport vehicles, lairages, and other sites (Annette *et al*., 2022).

Pathogenic Salmonellae consumed in food survive passage past the gastric acid barrier infiltrate the mucosa of the small and large intestines and create toxins. Invasion of epithelial cells induces the release of proinflammatory cytokines which generate an inflammatory reaction. The immediate inflammatory response causes diarrh~~o~~ea and may progress to ulceration and damage of the mucosa. The bacteria can disseminate from the intestines to produce systemic disease (Al-Seghayer and Al-Sarraj, 2021).

Salmonellosis in pig farms is widespread worldwide, producing illness and mortality and consequently, economic losses (Abiodun *et al*., 2014; Ahmed *et al*., 2017).

The rise of antibiotic-resistant *Salmonella* strains has heightened the worry of public health as these bacteria are more virulent, producing an increase in the mortality rate of infected patients (Wemedo *et al*., 2023). Due to the nature of piggery and its surroundings, the risks for contamination and cross-contamination resulting ~~to~~ insickness are relatively high (Chiu *et al*., 2002).Salmonellosis is associated ~~to~~ withthe intake of *Salmonella*-contaminated food products predominantly from poultry, pig, and egg products (Barika *et al*., 2023). Due to inadequate cleanliness procedures in the piggery environment in the Port Harcourt metropolis. Hence this research was carried out to investigate the prevalence and virulent factors of *Salmonella* spp in pigs and piggery in Port Harcourt metropolis.

MATERIALS AND METHODS

Collection and processing of samples

The study was carried out in two (2) different locations in Port Harcourt Metropolis, with seven (7) sampling points in each location. Sampling stations were M&K farms (Station A) the coordinates are Latitude 4.8854°N and Longitude 6.9249 °E, and Rivers State University pig farm (Station B) the coordinates are Latitude 4.8064 °N and Longitude 6.9864 °E. The choice of the study areas is due to the high piggery product consumption by ~~residence~~ residentsin these areas.

A total of 112 samples were collected for 2 months comprising of faecal, urine, and Swaps from the Pig food trough, water trough, floor, wall, and skin ~~were~~ was collected from the two separate locations and seven sampling points from each location in Port Harcourt Metropolis. The faecal sample were collected using the sterile spatula and placed in sterile sample containers, floor, foreskin, food trough, wall, and water trough samples ~~were~~ was collected by swabbing the surfaces with swab sticks containing prepared peptone water, and the urine samples were collected in sterile urine sample bottles. The samples collected were marked properly, placed in an ice chest, and transported aseptically to the Department of Microbiology, Rivers State University laboratory for bacteriological investigation.

Microbiological Analysis

Enumeration of Bacteria and Maintenance of Pure Culture of Bacteria

Serial tenfold dilution was conducted out on the weighed sample of faeces (1g in 9ml) and (1ml in 9ml) floor, feeding trough, foreskin, wall, and urine samples with a dilution ratio from 10^{-1} to 10^{-6} (Avishai and Charles, 2014). Aliquot (0.1ml) of appropriate dilutions were spread out in duplicates into *Salmonella-Shigella*Agar. The plates were incubated at 37^{o}C for 24 hours. The colonies grown on the plates were counted and recorded. Discrete colonies were described and sub-cultured into *Salmonella-Shigella* Agar and incubated at 37^{o}C for 24 hours to obtain a pure culture (Cheesbrough, 2006).The pure culture was stored in 10% (v/v) glycerol suspension at -4^{o}C to prevent damage ~~of~~ to the pure culture

Isolation and Identification of Salmonella spp

Salmonella spp isolates were isolated based on colonial/morphological characteristics such as size, colour, elevation, surface, and black center colony on the media which is the hallmark of the organism. Gram Staining, and Biochemical tests such as Tripple sugar iron Test, oxidase, Indole, Methyl red, Voges Proskauer, Glucose, Lactose, Mannose, Sucrose, and Citrate Utilization test were carried out to confirm *Salmonella* spp (Cheesbrough, 2006).

Test for Virulence

The virulence property of bacterial isolates was evaluated to identify the bacterial capacity to cause disease (Cha~~r~~kraborty and Nishith, 2008). The virulence factors evaluated are Haemolytic activity, motility, coagulase, and biofilm formation.

Data Analysis

Statistical analysis was carried out on the bacterial counts from the Piggery environment acquired in the study. Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) ~~was~~ were performed to test for significance and means separation between the Locations accordingly. This was done ~~utilising~~ utilizing a computer-based Programme-SPSS version 23.

Result

The result of the *Salmonella-Shigella*count of the pigs and Piggery obtained from stations A and B is presented in Table 1. The result of the analysis showed that the mean Total *Salmonella-Shigella* Count from station A ~~were~~ was feacal $2.3\pm0.5\times10^{5}$cfu/g, urine $5.0\pm0.1\times10^{3}$cfu/ml, floor $6.5\pm4.8\times10^{4}$cfu/m^{2}, food trough $2.4\pm0.3\times10^{4}$cfu/m^{2}, foreskin $4.1\pm0.4\times10^{4}$cfu/m^{2}, wall $4.6\pm0.5\times10^{4}$cfu/m^{2}and water trough $5.13\pm0.4\times10^{4}$cfu/m^{2}. While the mean *SalmonellaShigella* Count from station B ~~were~~ was feacal $2.5\pm0.52\times10^{5}$cfu/g, urine $0.0\pm0.0\times10^{3}$cfu/ml,floor $1.29\pm5.2\times10^{5}$cfu/m^{2}, food trough $7.9\pm5.1\times10^{4}$cfu/m^{2}, foreskin $5.0\pm0.7\times10^{4}$cfu/m^{2}, wall $9.0\pm6.6\times10^{4}$cfu/m^{2} and water trough $8.5\pm3.8\times10^{4}$cfu/m^{2}. The *SalmonellaShigella* bacterial load of the foreskin of the piggery for station B showed a significant difference in floor and food trough samples ($p>0.05$) higher than that of station A. The prevalence of *Salmonella* sppin pigs and Piggery is presented in Table 2 with faecal samples from both stations having the highest prevalence of 10.7%.

The result of some of the virulence tests performed on the *salmonella* isolates as shown ~~on~~ in ~~table~~ Table 3 indicates that all isolates tested were 100% positive ~~to~~formotility test, haemolysis, catalase, and S. *typhimurium*(80%), S. *choloraesius*(89.4%), S. *enterica* (93.3%)S.*enteritidis* (54.5%) and S. *bongori* (100%)to biofilm production test.

Table 1 Mean Salmonella-Shigella Count for Piggery Environment from Stations A and B

Sample	Unit	Station A	Station B	P-value
Fecal (fe)	$\times 10^5$cfu/g	2.3±0.5 [a]	2.5±0.5 [a]	0.396
Floor (fl)	$\times 10^4$cfu/m^2	6.5±4.8[a]	12.9±5.2 [b]	0.023
Food trough (ft)	$\times 10^4$cfu/m^2	2.4±0.3 [a]	7.9±5.1 [b]	0.021
Foreskin (fo)	$\times 10^4$cfu/m^2	4.1±0.4 [a]	5.0±0.7 [a]	0.762
Urine (u)	$\times 10^3$cfu/ml	5.0±0.1 [a]	0.0±0.0 [a]	0.207
Wall (w)	$\times 10^4$cfu/m^2	4.6±0.5 [a]	9.0±6.6 [a]	0.165
Water trough (wt)	$\times 10^4$cfu/m^2	5.13±0.4 [a]	8.5±3.8 [a]	0.126

Key: Means with similar superscripts across the rows showed no significant difference (P>0.05)

Table 2 Prevalence of *Salmonella* species from the various sources and locations

Source	Station A (M&K Pig Farm (%))	Station B (RSU Pig Farm (%))
Faecal	8(10.7)	8(10.7)
Floor	7(9.3)	7(9.3)
Food trough	6(8)	6(8)
Foreskin	5(6.7)	4(5.3)
Urine	3(4)	0
Wall	5(6.7)	6(8)
Water trough	5(6.7)	5(6.7)

Test	S. *typhimurium* n(%)	S. *choloraesius* n(%)	S. *enterica* n(%)	S.*enteritidis* n(%)	S. *bongori* n(%)
Heamolysis	26 (100%)	19(100%)	15(100%)	11(100%)	4(100%)

Table 3Virulent Test of the *salmonella* spp

Motility	26 (100%)	19(100%)	15(100%)	11(100%)	4(100%)
Coagulase	0	0	0	0	0
Catalase	26 (100%)	19(100%)	15(100%)	11(100%)	4(100%)
Biofilm production	21(80%)	17(89.4%)	14(93.3%)	6(54.5%)	4(100%)

Discussion

Salmonella is a major concern in ~~the~~ piggery due to its potential to cause severe illness in both humans and animals. The total *Salmonella-Shigella* count from station B~~were~~ waswas higher in feacal $2.5\pm0.5x10^5$cfu/g, floor $12.9\pm5.2x10^4$cfu/m^3, food trough $7.9\pm5.1x10^4$cfu/m^3, foreskin $5.0\pm0.7x10^4$cfu/m^3, wall $9.0\pm6.6x10^4$cfu/m^3 and water trough $8.5\pm3.8x10^4$cfu/m^3,and Station A urine $5.0\pm0.1x10^3$cfu/ml. This study reveals the high *salmonella* count recorded is due to faeces ~~are~~ beingin direct contact with the ground, which is naturally home to a variety of *salmonella* spp this direct contact allows for a transfer of bacteria to the floor, food trough, foreskin, wall and water trough and contributing to the higher counts. This study is in line with a study conducted by Jain *et al*., 2019. The *Salmonella-Shigella* bacterial load of the floor $12.9\pm5.2x10^4$cfu/m^3 from station B was significant ($p>0.05$) higher than those of station A $6.5\pm4.8x10^4$cfu/m^3. The presence of *Salmonella* spp in the pigs and piggery may be a result of contaminated water sources, and feed contamination. person-to-animal contact and improper cleaning process of the piggery (CDC, 2020).

The prevalence of *Salmonella* species from both stations sampled ~~were~~ washigh, this can be attributed to salmonella ~~been~~ beinga halo-tolerant. This studyhas identified pigs as carriers of *Salmonella*, with the bacteria commonly found in their intestines, and concord with a study by Ismail *et al*., 2012. Contaminated feaces, contaminated water, and contaminated feed can serve as sources of transmission, making piggeries a hotbed for *Salmonella* colonization. The warm and moist environment of the piggery creates a favorable habitat for bacterial growth and proliferation. Faecal samples are particularly conducive to bacterial growth, as they provide a nutrient-rich medium for bacteria to thrive(Zhang *et al*., 2018).

A study conducted in Malaysia found that 12.8% of the pig fecal samples collected from piggeries were positive for *Salmonella* (Ismail *et al*., 2012). Another study conducted in China reported a *Salmonella* prevalence rate of 10.1% in pig faeces samples collected from piggeries (Zhang *et al*., 2018). The prevalence of *Salmonella* species in piggery environments is a matter of concern due to its potential transmission to humans through the food chain.

Salmonella species are well-known for their pathogenicity and the ability to cause a range of diseases, including salmonellosis and typhoid fever in humans. The virulence of these bacteria is attributed to various factors, including haemolysis, biofilm formation, motility, and catalase activity.

*Salmonella*species possessing virulent properties are capable of causing disease conditions in pigs. ~~Result~~ The result of virulent ~~property~~ propertiesof *salmonella* species indicates that

hundred percent (100%) were positive ~~to~~ forMotility, Heamolysis, and Catalase, and 80.77% produced biofilm which is in agreement with (Barika *et al*., 2023).

~~Result~~ The result of the virulent propertyindicates that 100%of the *Salmonella* isolates were ~~Motile~~motile. *Salmonella* species have been extensively studied for their virulence using motility tests in various animal models, which have contributed to our understanding of their pathogenesis and potential interventions for controlling infections (Barika*et al*., 2023).

~~Result~~ The result of the catalase test shows that 100% were positive ~~to~~ forcatalase. Catalase is an enzyme that plays a crucial role in protecting bacterial cells from oxidative stress by catalyzing the decomposition of hydrogen peroxide into water and oxygen. *Salmonella* species have been shown to produce catalase, which can enhance their survival and persistence in the host environment by neutralizing the toxic effects of reactive oxygen species generated by the host immune system (Feng *et al*., 2015).

Hemolysis, the breaking down of red blood cells, can be influenced by the production of hemolysin by *Salmonella*. Hemolysin are toxins that can disrupt cell membranes, leading to the release of hemoglobin. This process enhances their virulence by allowing them to invade and damage host tissues more efficiently (Tatavarthy *et al*., 2014). The result shows that 100 % ~~0f~~ of salmonella isolates were positive ~~to~~ forHemolysis and this study is in concordance with (Barika *etal*, 2023)

Biofilm formation is another important virulence factor of *Salmonella* species. Biofilms are complex communities of bacteria that are encased in a matrix of extracellular polymeric substances and are known to be associated with increased antibiotic resistance and evasion of the host immune system. Eighty percent of *Salmonella* isolate tested were biofilms producers. Biofilm formation can facilitate their persistence in the environment and increase their capacity to cause infectious diseases, *Salmonella's* ability to form biofilms can contribute to its persistence and resistance to environmental stresses (Andrews *et al*., 2010).

The severity of *salmonella* infection in pigs depends on the virulent factor and the host's immune status. *Salmonella*~~are~~ispathogenic as they ~~have the ability to~~can invade, replicate, and multiply in a susceptible host cell, resulting ~~to~~ inpotentially fatal disease conditions (Hyeon *et al*., 2011).

Conclusion and Recommendation

In Conclusion, this study was able to determine the prevalence of *Salmonella*spp in pigs and piggery in Port Harcourt Metropolis with feacal sample having the highest prevalence of 10.7% and some of the virulence tests performed on the *salmonella* isolates ~~indicates~~ indicatingthat all isolates tested were 100% motile, haemolysis, catalase positive and S. *typhimurium* (80%), S. *choloraesius* (89.4%), S. *enterica* (93.3%)S.*enteritidis* (54.5%) and S. *bongori* (100%)produced biofilm.

Implementing strict hygienic measures in the piggery environment can help in preventing the introduction and spread of *Salmonella*. This includes proper sanitation, disinfection, and control of animals within the facility, use of personal protective equipment, and early detection of clinical signs.It is important to conduct regular monitoring and surveillance of the pigs and piggery for the presence of *Salmonella* species. This can be done through routine sampling of feacal matter, feed, water sources, and environmental surfaces. Educate, and train piggery personnel and the general public through advertisements and campaigns on the importance of *Salmonella* control and Keeping detailed records of *Salmonella* prevalence data to track trends over time and identify any emerging resistance patterns.

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