

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, 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 obtained with seven sample types comprising of faeces, floor, food trough, foreskin, urine, walls, and water trough were aseptically collected. Samples were examined for presence of *Salmonella* using standard microbiological approach for enumeration and identification. Mean *Salmonella-Shigella* counts (SSC) fecal sample ranges for station A and B were: 1.6 to 3.1×10^5 cfu/g, floor 5×10^4 to 2.0×10^5 cfu/m², food trough 3×10^4 to 1.2×10^5 cfu/m², Foreskin 4×10^4 to 1.4×10^5 cfu/m², urine 0 to 1×10^4 cfu/ml, Wall 5×10^4 to 2×10^5 cfu/m², Water trough 6×10^4 to 1.3×10^5 cfu/m². Seventy-five isolates of *Salmonella* belonging to 5 species were isolated which included with fecal 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. choleraesuis* (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 high potential risk of transmission of *salmonella* spp in piggery, which can have a serious implication to public health. It is essential that a more effective control strategies be employed to minimize the prevalence of *salmonella* spp in pigs and piggery in 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 diarrhoea 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 sickness are relatively high (Chiu *et al.*, 2002). Salmonellosis is associated to the intake of *Salmonella*-contaminated food products predominantly from poultry, pig, and egg products (Barika *et al.*, 2023). Due to inadequate cleanliness procedures in piggery environment in 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 in 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 collected from the two separate locations and seven sampling points from each location in Port Harcourt Metropolis. The faecal sample were collected using sterile spatula and placed in sterile sample container, floor, foreskin, food trough, wall, water trough samples were 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°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°C for 24 hours to obtain a pure culture (Cheesbrough, 2006). The pure culture was stored in 10% (v/v) glycerol suspension at -4°C to prevent damage of 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, black center colony on the media which is the hallmark of the organism. Gram Staining, and Biochemical tests such as Triple 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 (Charkraborty and Nishith, 2008). The virulence factor evaluated are Haemolytic activity, motility, coagulase, biofilm formation.

Data Analysis

Statistical analysis was carried out on the bacterial counts from Piggery environment acquired in the study. Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was performed to test for significance and means separation between the Locations accordingly. This was done utilising 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 faecal $2.3 \pm 0.5 \times 10^5$ cfu/g, urine $5.0 \pm 0.1 \times 10^3$ cfu/ml, floor $6.5 \pm 4.8 \times 10^4$ cfu/m², food trough $2.4 \pm 0.3 \times 10^4$ cfu/m², foreskin $4.1 \pm 0.4 \times 10^4$ cfu/m², wall $4.6 \pm 0.5 \times 10^4$ cfu/m² and water trough $5.13 \pm 0.4 \times 10^4$ cfu/m². While the mean *Salmonella-Shigella* Count from station B were faecal $2.5 \pm 0.52 \times 10^5$ cfu/g, urine $0.0 \pm 0.0 \times 10^3$ cfu/ml, floor $1.29 \pm 5.2 \times 10^5$ cfu/m², food trough $7.9 \pm 5.1 \times 10^4$ cfu/m², foreskin $5.0 \pm 0.7 \times 10^4$ cfu/m², wall $9.0 \pm 6.6 \times 10^4$ cfu/m² and water trough $8.5 \pm 3.8 \times 10^4$ cfu/m². The *Salmonella-Shigella* bacterial load of the foreskin of the piggery for station B showed significant difference in floor and food trough samples ($p > 0.05$) higher than that of station A. The prevalence of *Salmonella* spp in pigs and Piggery is presented in Table 2 with faecal samples from both station having the highest prevalence of 10.7%.

The result of some of the virulence test performed on the *salmonella* isolates as shown on table 3 indicates that all isolates tested were 100% positive to motility test, haemolysis, catalase and *S. typhimurium*(80%), *S. choleraesius*(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 Station A and B

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

Key: Means with similar superscript 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	<i>S. typhimurium</i> n(%)	<i>S. choleraesius</i> n(%)	<i>S. enterica</i> n(%)	<i>S. enteritidis</i> n(%)	<i>S. bongori</i> n(%)
Heamolysis	26 (100%)	19(100%)	15(100%)	11(100%)	4(100%)

Table 3 Virulent 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 higher in faecal $2.5 \pm 0.5 \times 10^5$ cfu/g, floor $12.9 \pm 5.2 \times 10^4$ cfu/m³, food trough $7.9 \pm 5.1 \times 10^4$ cfu/m³, foreskin $5.0 \pm 0.7 \times 10^4$ cfu/m³, wall $9.0 \pm 6.6 \times 10^4$ cfu/m³ and water trough $8.5 \pm 3.8 \times 10^4$ cfu/m³, and Station A urine $5.0 \pm 0.1 \times 10^3$ cfu/ml. This study reveals the high *salmonella* count recorded is due to faeces are in 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 $12.9 \pm 5.2 \times 10^4$ cfu/m³ from station B was significant ($p > 0.05$) higher than those of station A $6.5 \pm 4.8 \times 10^4$ cfu/m³. 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 high, this can be attributed to salmonella been a halo-tolerant. This study has 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 faeces, 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 of virulent property of *salmonella* species indicates that hundred percent (100%)

were positive to Motility, Hemolysis, Catalase and 80.77% produce biofilm which is in agreement with (Barika *et al.*, 2023).

Result of virulent property indicates that 100% of the *Salmonella* isolates were 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 of catalase test shows that 100% were positive to catalase. 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*. Hemolysins 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 100% of salmonella isolates were positive to Hemolysis and this study is in concordance with (Barika *et al.*, 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 immune status. *Salmonella* are pathogenic as they have the ability to invade, replicate and multiply in a susceptible host cell, resulting to potential fatal disease condition (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 fecal sample having the highest prevalence of 10.7% and some of the virulence test performed on the *salmonella* isolates indicates that all isolates tested were 100% motile, haemolysis, catalase positive and *S. typhimurium* (80%), *S. choleraesuis* (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 animal 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 fecal matter, feed, water sources, and environmental surfaces. Educate, train piggery personnel and the general public through advertisement and campaign 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.

References

- Al-Seghayer, M.S. and Al-Sarraj, F.M.B (2021). The Outbreak of Foodborne Disease by Pathogenic Enterobacteriaceae Antimicrobial Resistance. *Journal of Epidemiology and Infection*.20(6) 91-99
- Andrews, C. M., Girard, J. E., Hilsenbeck, J. L., and Pielsticker, J. L. (2010). In vitro biofilm formation and antibiotic resistance of *Salmonellaenterica* serovars *typhimurium* and *enteritidis*. *International Journal of Microbiology*. (2)1-7.
- Annette, D., Declan M., Finola C. L., William B., Tracey C., Gillian M., Margaret G., John E., and Deirdre M. P. (2022) Prevalence of *Salmonella* spp. in slaughter pigs and carcasses in Irish abattoirs and their antimicrobial resistance, *Irish Veterinary Journal* 75:4.
- Abiodun, A., Lloyd, W., Lisa, M., Bowen, L., George, J., and Alva, S.J. (2014). Resistance to antimicrobial agents among *Salmonella* isolates recovered from layer farms and eggs in the Caribbean region. *Journal of Food Protection* 77(12), 2153–2160.
- Ahmed, O.A., Mamman, P.H., Raji, M.A., and Aremu, A. (2017). Occurrence, genetic characterization and antimicrobial resistance of *Salmonella* isolated from chicken meat and giblets. *Journal of Epidemiology and Infection*. 14(3) 997–1003.
- Avishai, B. and Charles, E.D. (2014) Estimation method for serial dilution experiments, *Journal of Microbiological Methods*, 107, 214-221, ISSN 0167-7012.
- Barika, P.N., Akani, N.P., Amadi, L.O., and Sampson, T. (2023). Prevalence and antibiogram of *salmonellaenterica* isolated from seafood sold in Rivers State, Nigeria. *International Journal of Microbiology and Applied science*. 2;83-92
- Centers for Disease Control and Prevention. (2020) Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter – United States. *MMWR Morbidity and mortality weekly report*. 56:521–524.
- Charkraborty, J., Nishirt, M.P. (2008) Microbial ecology of foodborne pathogens associated with produce. *Current Opinion in Biotechnology*. 2(1), 125–130.
- Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. Part 2, 2nd Edition, Cambridge University Press Publication, South Africa. (6) 1-434

- Chiu, C.H, Wu, T.L., Su, L.H., Chu, C., Chia, J.H., Kuo, A.J., Chien, M.S., and Lin, T.Y. (2002). The emergence in Taiwan of fluoroquinolone resistance in *Salmonella enterica* serotype *choleraesuis*. *The New England Journal of Medicine*. 346:413–419.
- Crump, J.A., Sjölund-Karlsson, M., Gordon, M.A., and Parry, C.M. (2016). Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance and antimicrobial management of invasive *Salmonella* infections. *Clinical Microbiology Review*. 2(8) 901–910.
- Feng, Y., Mao, Y., Qiao, L., Chen, D., and Wu, X. (2015). Catalase and Alkyl hydroperoxide Reductase Have Positive Effects on the growth of *Salmonella enterica* Serovar *typhimurium*. 10(12), 1-13.
- Hyeon, J.Y., Chon, J.W., Hwang, I.G., Kwak, H.S., Kim, M.S., Kim, S.K., Choi, I.S., Song, C.S., Park, C., and Seo, K.H. (2011). Prevalence, antibiotic resistance, and molecular characterization of *Salmonella* serovars in retail meat products. *Journal of Food Protection*. 74; 161-166.
- Ismail, N., Hussin, S., and Darus, S. (2012) Molecular epidemiology of 3 household transmissions of *Salmonellatyphi* in Kelantan, Malaysia. *International journal of infectious diseases*. 5(3) 78-89.
- Jain, S., Mukhopadhyay, K., and Thomassin, P.J. (2019) An economic analysis of *Salmonella* detection in fresh produce, poultry, and eggs using whole genome sequencing technology in Canada. *Food Research International*. 11(6), 802–809.
- Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A., and Hoekstra, R.M. (2010). The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clinical Infectious Diseases*. 50(6):882–889.
- Tatavarthy, A., Cannis, P., Holder, A., and Rangaswamy, D. (2014). Haemolytic Uremic Syndrome Induced by *Salmonella*. *Case Reports in Hematology*. (4) 1-4.
- Wemedo, S.A., Williams, J.O., and Ndem, D. L. (2023). Prevalence and antimicrobial susceptibility pattern of *salmonella* among food and food vendors in Port Harcourt, Nigeria. *South Asia Journal of Research in Microbiology*. 15(3) 21-29.
- Zhang, Y., Xin, Y., Hongwei, Z., Yongheng, B., Youzhi, L., Yang, L., Jianlong, Z., Guozhong, C., and Xingxiao, Z. (2018). Prevalence and antimicrobial resistance of *Salmonella enterica* subspecies *enterica* serovar *enteritidis* isolated from broiler chickens in Shandong Province, China. *Journal of Poultry Science*(2): 1016–1023.