Original Research Article

Assessing Environmental and Feed Quality in Southeast Livestock Production

ABSTRACT

Livestock production has thrived and sustained the livelihood of farmers while reducing poverty and improving the economy of developing nations. It has also contributed largely to the spread of pathogenic bacteria. This study evaluated the bacterial quality of air, water, soil, feed samples, and hand swabs in Aba, Umuahia, Mbaise, and Okigwe inSoutheastern Nigeria. Air, water, soil, feeds, and hand swabs were sampled using standard microbiology laboratory procedures. Air samples were expressed in CFU/plate/hour while water, soil, feed and hand swabs were expressed in CFU/ml. Total heterotrophic bacterial count (THBC), total coliform count (TCC), and total potential pathogenic bacterial count (TPPBC) were all analyzed by growing the samples on general purpose, differential, and selective media after serial dilution respectively. Isolates were confirmed by subjecting them to biochemical tests. The highest THBC, TPPBC, and TCC values were obtained in the Aba pig farm, cow abattoir, and poultry farms respectively while Mbaise recorded the lowest values for THBC, TPPBC, and TCC. A total of 1531 bacterial isolates were obtained in all the four cities. *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Salmonella enterica, Streptococcus poygenes, Vibrio cholerae* and *Shigella* sp. were isolated. *Escherichia coli* had the highest total percentage distribution (16.1%; 259/1531) in all four cities and *Shigella*sp (4.8%; 74/1531) was the least distributed. Of the four cities studied, Aba farm had the highest percentages of *E. coli* (31.67%; 82/259), *K. pneumoniae* (33.67%;68/218), *P. aeruginosa* (30.28%; 43/31.85%), *E. aerogenes* (31.85%; 43/135) and *S. pyogenes* (36.57%; 49/134). Among the bacterial isolates, *Streptococcus enterica* had the highest percentage values (42.38%; 64/151) while *Shigella*sp had the lowest percentage values (12.16%; 9/74). Overall, the bacterial burden in all the farms in the four cities, especially Aba was very high; thorough surveillance by health workers should be encouraged to help reduce the spread of bacteria and bacterial infections among livestock farms.

1.0 INTRODUCTION

Livestock production as practised globally has thrived and sustained the livelihood of farmers while reducing poverty and improving the economy of developing and underdeveloped countries (Upton, 2016). In developing countries, cows, goats, pigs, and poultry are the commonly reared animals. These animals are managed for meat, skin, milk, and egg production for domestic consumption and industrial purposes (Steinfeld et al., 2006). While livestock farming has helped to improve the economy, it has unfortunately heightened the level of microbial loads in the environment, which has directly or indirectly affected the health conditions of the people (Manyi-Loh et al., 2018).

Nigeria is a developing country with people from different diverse cultural backgrounds. It is estimated that before 2040, the population will rise four times the current assumed figure of 200 (Asadu et al., 2021). Due to geopolitical arrangement, the country is grouped into six zones namely Southeast, Southsouth, Southwest, Northeast, Northwest, and Northcentral. Each of these zones has its peculiarities in terms of economy and agriculture, especially livestock farming. The Southeast region has 5 states and is known for its commercial and agricultural activities. Due to food insecurity, and poverty, people from this zone living in rural areas moved to urban centres to engage in livestock farming (Asadu et al., 2021; Mouratidis, 2019). Livestock farming in Southeast Nigeria aside from reducing hunger and poverty has contributed negatively to public health (Nwokoro et al., 2017).

Bacteria are ubiquitous and are capable of surviving on farms and could further spread to farmers through direct contact with infected animals, unhygienic practices of farmers and the consumption of undercooked meats (Rayman et al., 2020). Furthermore, while it is advantageous using the droppings of this livestock as a source of manure to improve soil fertility, they could be a source of bacterial contamination (McAllister & Topp, 2012). Therefore, absolute caution should be taken before any agricultural practices are considered. Bacteria from these dropping could percolate to nearby rivers during rain splash (Agodo et al., 2016). These bacterial cells could be pathogenic to humans and might surreptitiously affect the skin and some vital organs (Klous et al., 2016). Diseases produced from these farms and abattoirs through the spread of bacteria affect urban dwellers through air, water and meat consumption (WHO, 2018),

especially people living close to the farms. This study shall evaluate the bacterial loads in farms and abattoirs in the Southeast, using Aba, Umuahia, Okigwe, and Mbaise as case studies.

METHODS

Study area

Aba and Umuahia in Abia State and Okigwe and Mbaise in Imo State were selected based on the rapid livestock activities taking place there. The samples were collected from urban areas where livestock farming is vigorously practised. These four cities stretch from latitude $4^{o}50'$ to $7^{o}20'$ N and longitude $6^{o}51'$ to $8^{o}20'$E. It has a common boundary with Benue State in the North, in the East it is bounded by Cross River and Akwa Ibom States, in the West by Delta State and River Niger (Kalu &Zakiora, 2019). The zone has diverse ecological variations and a land mass of 22,525 km^2 (Madu, 2006). Its annual rainfall is between March and October while the dry season starts in November and ends in February (Kalu &Zakiora, 2019). The study was carried out from December 2019 to April 2022. A total of 600 samples (air, water, soil, feeds) were collected from pig and poultry farms and cow abattoir including hand swabs of their keepers. Feeds were obtained aseptically only from poultry and pig farms in Aba, Umuahia, Okigwe and Mbaise while air, water and soil samples were collected aseptically from pig and poultry farms and cow abattoir in the four cities of the two states using scientific standards. Hand swabs of the livestock workers were also collected with sterile swab sticks in all the farms.

Environmental samples collection

Air from the pig, poultry farms, and cow abattoir were sampled using the settle plates technique. Newly prepared selective and differential media plates such as blood agar (BA), Salmonella-Shigella agar (SSA), MacConkey (MCA), eosin methylene blue agar (EMB), mannitol salt agar (MSA) and Thiosulphate citrate bile salt sucrose (TCBS) plates were exposed to the air at different locations of the farms and abattoir at approximately 1.5 m height for 1 h. the sample collection was repeated three times. Also, nutrient agar plates were exposed at the same height for the total heterotrophic bacterial count. All the plates were carefully covered, labelled, and transported to the laboratory in a polyethene bag. The plates were returned to the incubator for the growth of bacteria at $30^{o}C$ for 24 h. The livestock's drinking water

was sampled with transparent sterile flasks (5 litres) and transported to the laboratory for analysis (Ugbogu*et al*., 2016). Ten-fold serial dilution of the water samples collected from Aba, Umuahia, Okigwe and Mbaise was performed according to the method described by Harley and Prescott (2002). Soil samples were collected using a soil auger at a 5 cm depth (Bhat *et al*., 2011) where microbial population and activities take place. From different locations in the pig farm, poultry farm and cow abattoir, 50 g of each soil was collected, transferred into a black polyethene bag, labelled and transported under cold conditions (4 ± 2 ◦C) to the laboratory for analysis. Hand swabs from cow butchers, and pig and poultry farm workers were also collected using sterile swab sticks. Twenty grammes of feed samples from all the farms in the four cities were collected in plastic bags straight from the feed containers and transported to the laboratory.

Laboratory analysis

Soil, feed and water samples aseptically collected were analysed using a 10-fold dilution (10^{1}-10^{7}). Briefly, 10 g from each soil sample was dissolved in 90 ml of distilled water. After proper mixing by constant shaking, 10 ml from the first tube containing the dilution factor was transferred aseptically to the next tube and continued till the last tube, after which 10 ml was discarded (Adhikari *et al*., 2007). The same procedure used for the serial dilution of soil was used for water analysis except that 10 ml from each water sample was serially diluted. For hand swab analysis, each labelled swab stick was placed into 7 ml of peptone water and allowedfor 10 min (Sampson *et al*., 2019). Thereafter, the solution was serially diluted between 10^{-1} and 10^{-4}. The serial dilutions of soil, feeds, water and hand swabs were repeated three times.

Bacterial enumeration

Total heterotrophic bacterial count (THBC)

Briefly, 1 ml from each of the fourth dilution tubes was aseptically transferred to nutrient agar plates with a pasture pipette. The discrete colonies on each nutrient agar plate exposed to air and inoculated with drops of solutions from water, feed and hand swab samples respectively between ≥30 and ≤ 300 were counted and recorded. The colonies counted represent the heterotrophic bacterial count (THBC).

Total potential pathogenic bacteria

Briefly, 1 ml from the fourth tube of water, feed, hand swabs, and soil dilutions were transferred aseptically to the already prepared blood, MSA, MacConkey, EMB, SSA, TCBS agar plates with sterile Pasteur pipette. The microorganisms suspected in the plates include Streptococcus aureus, *S. aureus*, E. coli, E. aerogenes, *Vibrio cholera* and *Vibrio parahaemolyticus* respectively. The inoculum from each blood and MSA agar plates was subjected to catalase and citrate biochemical tests. For air samples, the selective media plates earlier mentioned were exposed for a period of 1 h at approximately 1.5 m high. After incubating all the plates for a period of 24 h at 30^{o}C, the discrete colonies were counted with a hand lens and reported as TPPBC. The colony from each plate was identified by their cultural and morphological characteristics and further confirmed by chemical tests (Lama *et al.*, 2013).

Total Coliform Bacterial Count (TCC)

Water samples

Briefly, 50 ml from the fourth dilution tube after 10-fold serial dilution was transferred aseptically against a membrane filter (0.45 µm pore size). After percolation of the filtrate, the filter paper was carefully placed on the already prepared MacConkey agar plates with the help of a pair of sterilized tweezers. After incubating the plates for 24 h at 30^{o}C, the colonies were counted with the help of a hand lens and presented as TCC (Harley and Prescott, 2002).

Soil samples

After serial dilution, 50 ml solution from the fourth dilution tube was transferred aseptically against a membrane filter with a pore size of 0.45 µm. After the filtration process, the filter paper was laid onto the MacConkey agar plate, already prepared. The plates were incubated for 24 h at 30^{o}C. The inoculums

from the MacConkey agar plates were transferred aseptically into tubes containing 10 ml of lactose bile broth (Adhikari *et al*., 2007). After incubation for 24 h at 30^{o}C, the mixture was observed for fermentation.

Hand swabs

For enumeration of TCC of hand swab samples, 1 ml from the fourth dilution tubes were transferred directly to the MacConkey agar plates and spread uniformly on the plates with bent glass rod. The plates were incubated for 24 h at 30^{o}C.

Identification and characterization of bacterial isolates.

All the bacterial isolates were identified using their cultural characteristics, including elevation, margin, colour, size and surface texture. The Gram stain was conducted on all the bacteria and microscopically examined to differentiate between Gram-positive and Gram-negative bacteria. Furthermore, the isolates were subjected to biochemical tests (Onyeagba 2015). The results were compared with standard reference of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibons, 1974).

Pure culture

Each bacterial isolate biochemically confirmed was sub-cultured and transferred to already prepared nutrient agar slants in Makati bottles by streaking the media surfaces. The slant bottles were incubated at 30^{o}C for 24 h. After incubation, the slant bottles were kept in the refrigerator at -4^{o}C for storage for further use.

Statistical analysis

The mean in triplicates was expressed as mean± SD; two-way ANOVA compared the means followed by Bonferroni post hoc test using graph pad prism graphical statistical package version 5.

3.0 RESULTS

Bacterial profile of the Pig, Poultry farms, and Cow abattoir in Aba

The THBC in the Aba pig farm obtained from soil samples was significantly higher than those obtained from air and hand samples. In the pig farm, the TPPBC of soil samples was significantly higher than other samples while drinking water had the lowest values. The highest values of TCC was seen in feeds while hand swab had the lowest values. Drinking water had the highest TCC while hand swabs had the least. In the cow abattoir, the highest value of THBC was seen in soil samples while air (18.56±0.3) has the lowest. In the study, the THBC, TPPBC and TCC of drinking water and feeds were not determined in cow abattoir. The TCC in cow abattoir was significantly higher in soil samples than hand swabs. In poultry farm, the THBC of soil samples was significantly higher than the values obtained in drinking water, hand swabs and feeds while the air samples of poultry farm had the highest values, and drinking water had the lowest. were higher. The TCC of samples was higher than in drinking water and hand swabs. The feed samples had the least values of TCC. In all the two farms and cow abattoir, The TCC were not determined. In all the farms and cow abattoir examined, pig farm had the highest values of THBC, TPPBC, and TCC. The bacterial loads of pig and poultry farms and cow abattoir are presented in Figure 1.

Bacterial profile of the Pig and Poultry farms and Cow abattoir in Umuahia

The THBC of soil in pig farm is significantly higher than in feeds, hand swabs, drinking water, and air samples with hand swabs having the least values. The soil samples had appreciable values of TPPBC and TCC than the other samples studied while drinking water and hand swabs had the lowest values of TPPBC and TCC respectively. In cow abattoir, the THBC of soil samples was significantly higher than in air and hand swabs. For feed and drinking water, the TPPBC and TCC were not determined. Whereas the TCC of soil samples was significantly higher than hand swab, TCC for drinking water, air and feeds were not determined. The THBC of soil samples was significantly higher than in feed, hand swab, drinking water, and air samples. In poultry farm, the lowest value was obtained from air samples. The

TCC of air samples was not determined. Among the three farms evaluated, pig farms had the highest THBC, TPPBC and TCC while cow abattoir had the lowest values for THBC, TPPBC and TCC (Figure 2).

Bacterial profile of the Pig and Poultry farms and Cow abattoir in Okigwe

In pig farms, the THBC and TPPBC of soil are significantly higher than in feeds, hand swabs, drinking water, and air samples. The hand swabs had the lowest value of THBC and TPPBC. The TCC of feeds was significantly higher than the other samples with hand swabs being the lowest. In cow abattoir, the soil samples had significant values of THBC, TPPC and TCC while air samples hand swabs had the lowest values of THBC and PPBC and TCC respectively. The THBC, TPPBC and TCC of drinking water, as well as the TCC of air samples, were not determined. In poultry farms, the THBC and TCC of soil samples were significantly higher than those obtained from hand swabs, drinking water and air samples while the TPPBC of air samples were significantly higher than samples. THBC of air samples had the lowest values while TPPBC and TCC of drinking water and hand swabs respectively had the lowest values. The TCC of air samples was determined. Among the three farms, cow abattoir had the highest values of THBC while poultry farms had the highest values of TPPBC and TCC. The bacterial profile of the pig and poultry farms and cow abattoirs in Okigwe is presented in Figure 3.

Bacterial profile of the Pig and Poultry farms and Cow abattoir in Mbaise

In poultry farms, the THBC and TCC were significantly higher in soil samples when compared with other samples. Air had the highest values of TPPBC. The lowest values of THBC, TPPBC and TCC were obtained from air, drinking water and feeds respectively. The TCC of air samples were not determined in poultry farms. In the cow abattoir, the THBC, TPPBC and TCC of soil samples were significantly higher than hand swabs which had the lowest values. For drinking water samples, no THBC, TPPBC or TCC were determined. The TCC of air samples were not determined also. The THBC of pig farms were significantly higher than those obtained in other farms, while, hand swabs and drinking water produced the highest TPPBC and TCC values respectively. The lowest values of TPPBC and TCC were obtained in drinking water and feeds. The TCC of air samples was not determined. Cow abattoirs had the lowest

values of THBC, TPPBC and TCC. Among the three farms evaluated, poultry farms had the highest THBC, and TCC while pig farms had the highest values of TPPC (Figure 4).

Percentage distributions of bacterial isolates in Aba, Umuahia, Okigwe and Mbaise

Aba farm had the highest percentage of *E. coli* (31.67%; 82/259), *K. pneumoniae* (33.67%;68/218), *P. aeruginosa* (30.28%; 43/31.85%), *E. aerogenes* (31.85%; 43/135) and *S. pyogenes* (36.57%; 49/134); Umuahia had the highest percentage of *S. aureus*. *Vibrio cholerae* and *Shigella*sp were prevalent in Okigwe while *S. enterica* was highest in Mbaise. Among the bacterial isolates, *S. enterica* had the highest percentage value (42.38%; 64/151). The *E. coli* (259) had the highest distributions in all the four cities while *Shigella*sp (74) had the lowest distribution (Table 1).

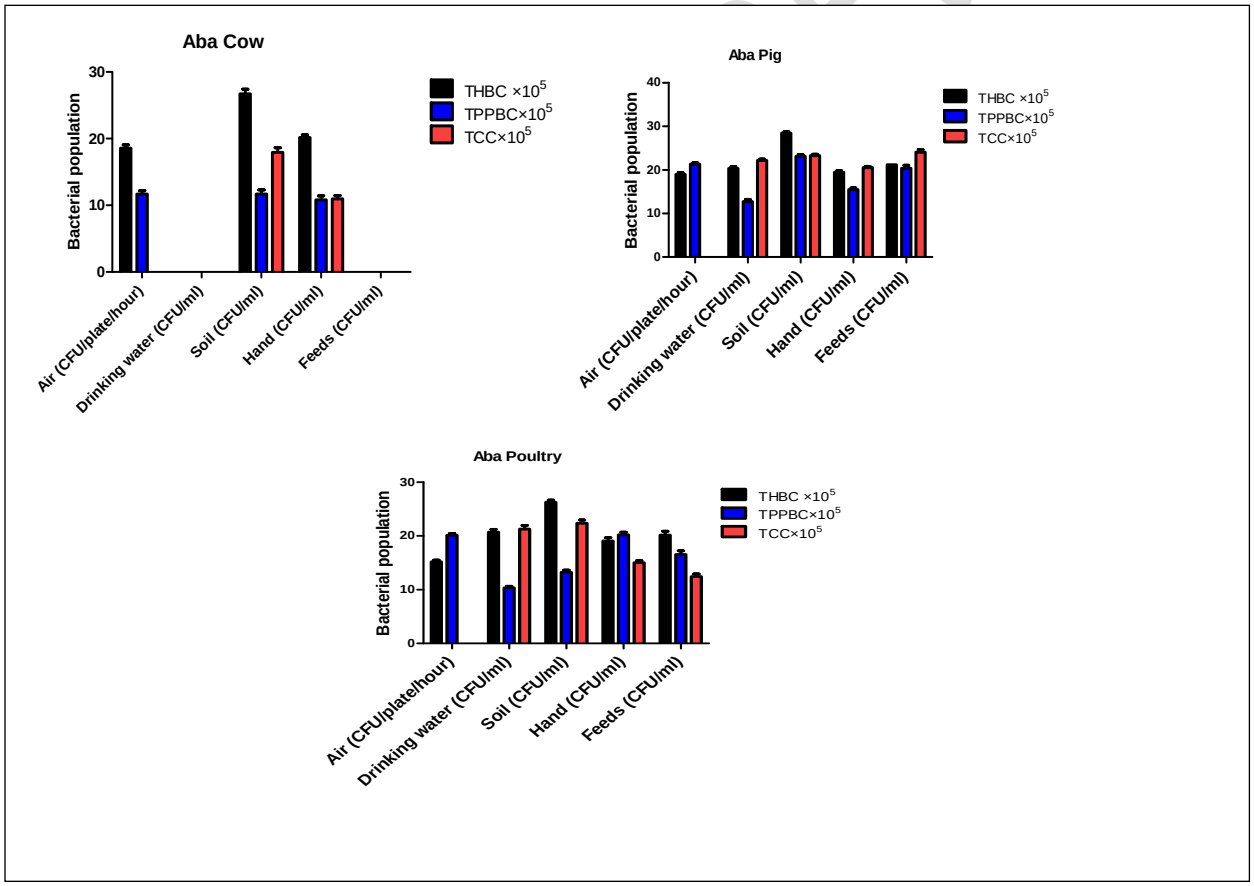

Figure 1: The THBC, TPPBC and TCC obtained from pig, poultry farms and cow abattoirs in Aba.

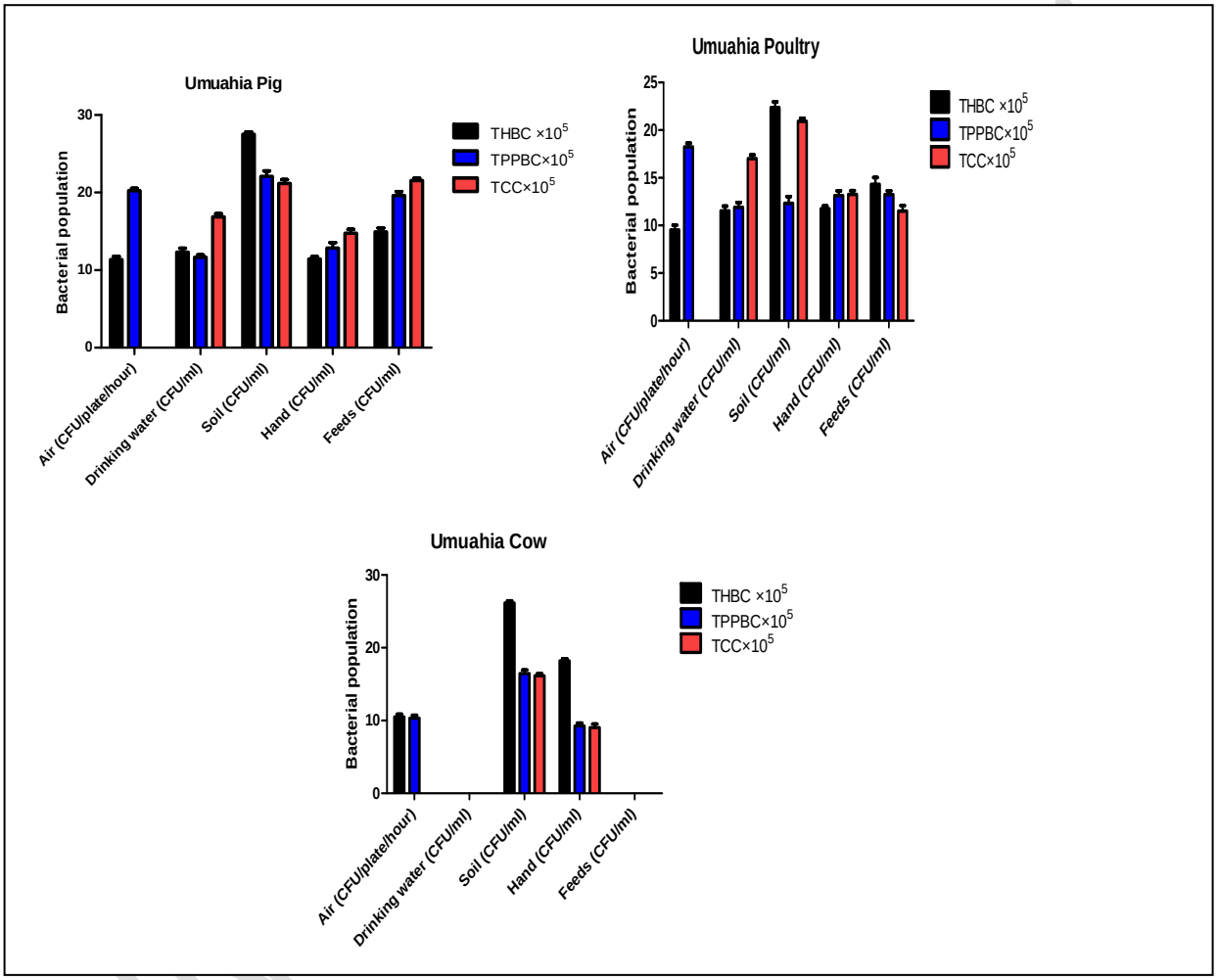

Figure 2: The THBC, TPPBC and TCC obtained from pig, poultry farms and cow abattoirs in Umuahia.

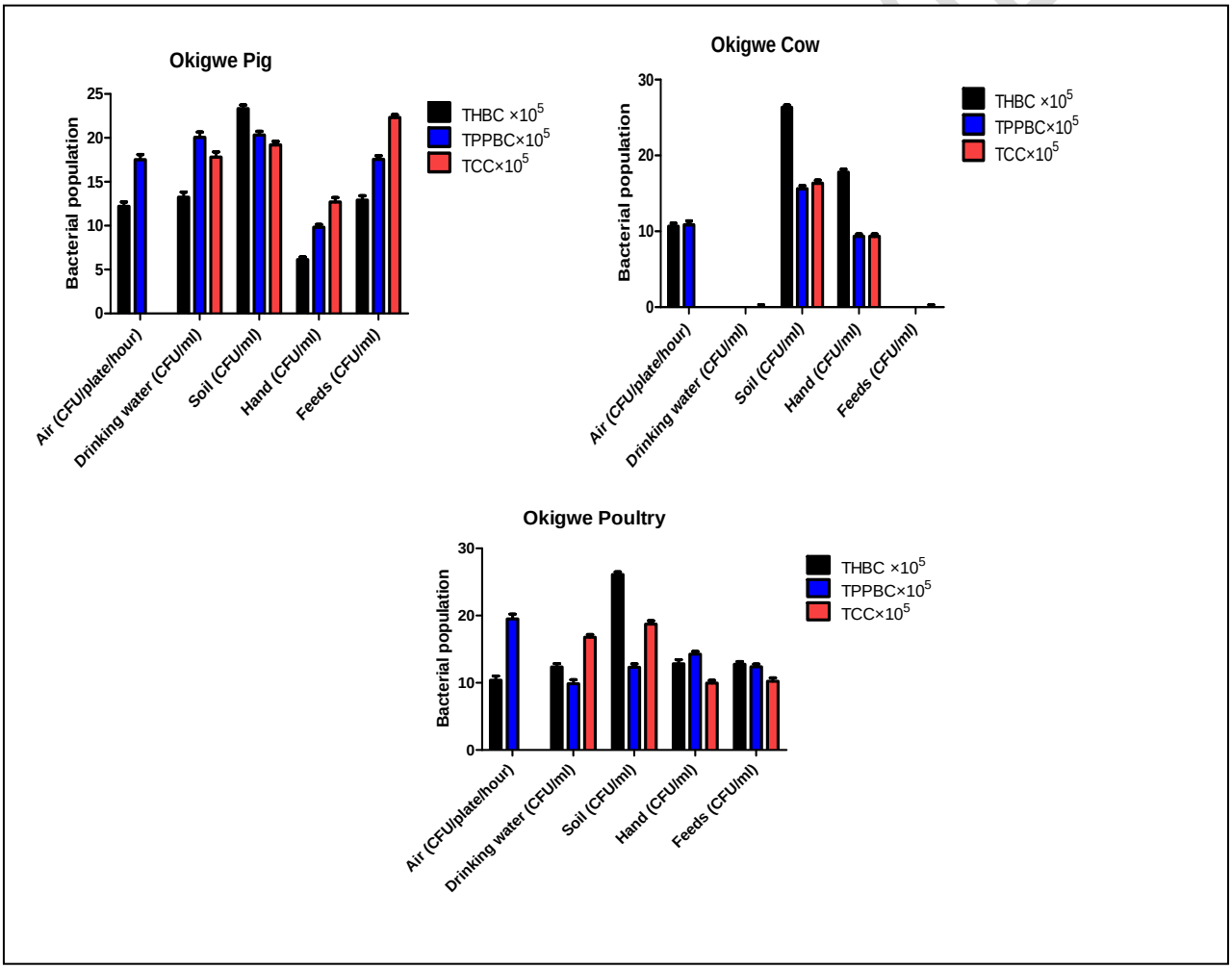

Figure 3: The THBC, TPPBC and TCC obtained from pig, poultry farms and cow abattoirs in Okigwe

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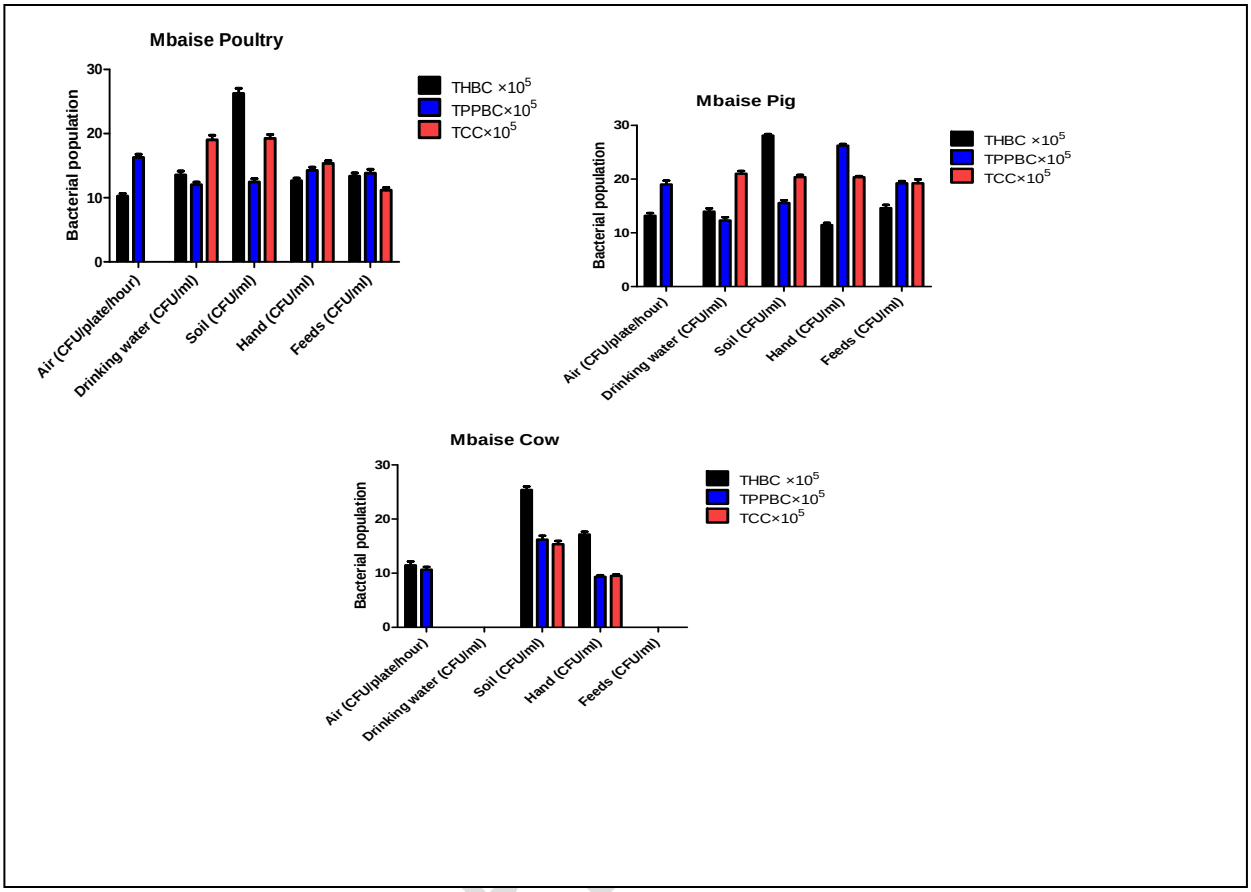

254 Figure 4: The THBC, TPPBC and TCC obtained from pig, poultry farms and cow abattoirs in Mbai

255 **Table 1:** Percentage occurrence of bacteria in Aba, Umuahia, Okigwe and Mbaise

Bacteria	Aba	Umuahia	Okigwe	Mbaise	Total
Escherichia coli	82 (31.67%)	45 (17.37%)	76 (29.34)	56 (21.62)	259 (100%)
K. pneumoniae	68 (33.67%)	45 (20.64%)	47 (21.56%)	58 (26.60%)	218 (100%)
S. aureus	56 (27.72%)	62 (30.69%)	54 (26.73%)	30 (14.85%)	202(100%)
Pseudomonas aerogenes	43 (30.28%)	35 (24.65%)	ND	32 (22.53%)	142 (100%)
S. enterica	54 (35.76%)	18 (11.92%)	15 (9.93%)	64 (42.38%)	151 (100%)
E. aerogenes	43 (31.85%)	23 (17.04%)	27 (20.00%)	42 (31.11%)	135 (100%)
Bacillus subtilis	12 (14.81%)	32 (39.51%)	14 (17.28%)	23 (28.39%)	81 (100%)
Vibrio cholerae	48 (35.56%)	ND	55 (40.74%)	32 (23.70)	135 (100%)
Streptococcus pyogenes	49 (36.57%)	ND	37 (27.61%)	48 (35.52%)	134 (100%)

Shigella sp.	9 (12.16%)	15 (20.27%)	25 (33.70%)	25 (33.70%)	74 (100%)

Discussion

Livestock farming has tremendously improved the livelihood of the populace, it creates opportunities for farmers to thrive as well as consumers of have value for their money. Figures 1, 2 and 3 expressed the THBC, TPPBC, and TCC of pig and poultry farms and cow abattoirs in Aba, Umuahia, Okigwe and Mbaise. The THBC has the highest microbial load when compared to others. The high values of THBC which are significantly different than TPPBC and TCC are as a result of the ubiquity of bacteria and their ability to survive in water (Nwaiwu et al., 2016), soil and air. Through frequent contact of feeds with the farmers and the unhygienic nature of the farms, microorganisms can survive and spread. Of all the farms, pig farm had the highest THBC; the THBC in pig farms is significantly higher than in poultry farms and cow abattoir. Pig farms are rendezvous of ammonia and other toxic chemicals like hydrogen sulfide that affect delicate regions of the human body; these wastes released from urine by these pigs and their faeces. When the waste water and the faeces are not properly disposed, they contribute to heterotrophic bacteria count (Samarin et al., 2021). Because the water, soil and air within pig farms are not taken care of by the farmers, therefore they could be harbingers of bacteria (Andretta et al., 2021).The soil is considered a good reservoir of microorganisms; it possesses water, nutrients, and protections for the sustenance of microorganisms.

The percentage distribution of *E. coli* in all four cities in Table 1 is predominantly higher than the other bacterial isolates. *Escherichia coli* is a Gram-negative bacterium which served as an indicator organism, especially in water and feeds. It measures the level of contaminants in faecal droppings due to unhygienic practices (Carvalho et al., 2018). The high values recorded could be a result of the warm weather conditions experienced in these parts of the country which makes the bacterium thrive (Nwaiwu et al., 2016). Olorunleke et al. (2022) asserted that the widespread *E. coli* strains in Southeastern Nigeria could be due to resistance to antibiotics. There might be other strains of *E. coli*, going forward that might be responsible if molecular studies were conducted (Carvalho et al., 2018). In the study, the bacterial population in Aba was more than in the other cities. *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E.*

*aerogenes*and *S. pyogenes* were prevalent in Aba. Aba is a cosmopolitan city, with rich commercial activities (Federal Ministry of Works., 2021); taking advantage of this, people from all walks of life flood into the city for livestock farming and other activities. Apart from improper waste disposal (Tolera et al., 2022), in other to feed the rising population, several sharp practices in the farms and abattoirs are performed. They are, the use of antibiotics in feeds for quick growth which inadvertently produced resistant bacterial strains (Amaechi, 2014, 2015), and the selling of meat infected with bacteria or their dead animals. These are done to the detriment of the consumers. These illicit activities increased the microbial loads within the city, especially in areas close to the farms. The high percentage of *S. aureus*, *S. enterica* and *V. cholerae* recorded in Umuahia, Mbaise, and Okigwe could be a result of unhygienic conditions of the farms and abattoirs in the southeast, faecal contaminants (Chibuzo & Okike, 2021), the lack of basic amenities in the processing of meat and inappropriate disposal of livestock solid and liquid wastes (Kalu, 2019). *S. aureus* is resident on skin surfaces and can spread via direct contact with livestock. *S. enterica* can be transmitted through the fecal-oral route which might endanger the lives of meat and egg consumers (Chibuzo & Okike, 2021).

Shigella species is a Gram-negative bacterium often isolated from poultry farms. It is responsible for causing shigellosis and cross-contamination in humans (Shi et al., 2014). In the study, it is the least isolated bacterium in the four cities. The low value recorded is unprecedented. As a developing nation coupled with the unhygienic practices of the southeasterners regarding livestock farm management, the values should have been higher than we had. The low result could be because only a few sites of poultry farms were assessed for the study. Meanwhile, the only city that had the highest percentage prevalence of *Shigella* was Okigwe; this is because Okigwe had more poultry farms than the other three cities. Apart from the multiple farms, the city is known for its polluted water due to its topography.

CONCLUSION

Livestock farming, despite being a source of job creation and food production has contributed to the spread of diseases. Microorganisms spread easily through water, air, direct contact with infected animals and animal feeds. By drinking contaminated water and consuming infested meat, the causative bacteria thrive. From the study, Aba recorded the highest THBC, evidence of an increased bacterial burdens. The

E. coli as seen in the study was the most distributed in the four cities which suggest poor hygienic practices among the farmers and butchers. In order to reduce the bacterial burdens in cities, government agencies should ensure total compliance to standard practices by farmers especially in the abattoirs where the meat is directly sold to consumers.

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