Detection of Virulence Determinants and Antibiogram of Bacteria Isolated from Semi-batch Digesters Treating Animal Manure

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Abstract: One of the drawbacks of land application of digestate is the possibility to contaminate the environment with potentially pathogenic, virulent and antibiotic resistant bacteria. The present study examined specifically the occurrence of virulence markers, haemolytic patterns and antibiotic susceptibility profiles of putative bacterial pathogens encountered during anaerobic digestion of manure before possible land application. Digestates collected from lab-scale semi-batch type anaerobic digesters were evaluated for the detection and occurrence of virulent and antibiotic resistant bacterial pathogens using standard microbiological methods. A total of 75 bacterial isolates were identified from poultry and goat manure samples, which belonged to 10 genera consisting of 40 (53.3 %) gram-positive bacteria (GPB) and 35 (46.7 %) gram-negative bacteria (GNB). Out of the 75 bacterial isolates from both samples, *Clostridium* sp, *B. subtilis* and *E. coli* had the highest frequency of occurrence 10 (13.3 %) each, closely followed by *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* 8 (10.6 %) each, *Staphylococcus aureus* 7(9.33 %), *Vibrio cholerae and Salmonella* sp 6(8.00 %) each, while *E. faecium* and *Shigella* sp had the least frequency of occurrences 5(6.66 %) each. The percentage occurrence of virulence attributes among encountered isolates were: 54.7% lecithinase; 56.0% gelatinase; 42.6% caseinase, and 52.0% amylase while haemolysin production were 35.0%, 29.3% and 36% for α-, β- and γ- haemolysis respectively. Among them, *Bacillus* species and *Pseudomonas aeruginosa* exhibited more virulence determinants. The susceptibility pattern of GPB and GNB showed 100 % sensitivity to ciprofloxacin, however, GPB displayed varied level of 100 % sensitivity to a single antibiotics ranging from two (2) antibiotics in *S. aureus* to eight (8) antibiotics each in *B. subtilis* and *Clostridium* sp. The survival and persistence of potentially pathogenic and virulent bacteria with antibiotic resistance traits is of public health significance. Hence, the need for continuous monitoring and microbiological evaluation of anaerobic digestate is highly recommended before application on arable land.

Keywords: Virulence determinants, antibiogram, digestate, pathogens, occurrence, manure

INTRODUCTION

The efficient management of livestock manure remains critical, as it poses a potential public health and environmental hazard (Qi *et al.,* 2019). This is a consequence of population growth across the world that has led to increasing environmental contamination/pollution due to humongous waste generation. Anaerobic digestion (AD) provides a promising route of treating organic waste such as livestock waste with cogeneration of biogas a renewable energy source, and digestate an enriched organic remains in the digester with biofertilizer potential (Ndubuisi-Nnaji *et al.*, 2020a). However, after AD, the existence and high concentrations of indicator bacteria, potential pathogens, heavy metals etc, in anaerobic digestate have been reported by researchers (Qi *et al.,* 2018, Resende *et al.*, 2014; Thomas *et al.,* 2019). The assessment of livestock manure digestate for bacterial indicators and pathogens becomes imperative to ensure environmental protection and safety of humans/animal.

Goat manure is an excellent raw material for anaerobic digestion because of its high total nitrogen content, fermentation stability and insensitivity to acidification during anaerobic fermentation (Zhang *et al.,* 2013). More so, poultry manure is a nitrogen rich biofertilizer, which is usually recycled and spread on agricultural fields. However, when untreated chicken litter is applied in its native form, concerns raised may stem from its height content of antibiotic resistant pathogenic

bacteria (Anjum *et al.,* 2016). In Akwa Ibom State and many parts of Nigeria, livestock manure especially poultry and goat manure are usually applied in its raw or untreated form as biofertilizer for the cultivation of fluted pumpkin, cucumber, garden egg and many other crops (Idem *et al.,* 2012). However, little attention has been given to the chemical and microbiological quality status of these manures. It has been recognized that waste generated from livestock production has great potential for environmental degradation (Coelho *et al.,* 2018). Livestock manure is one of the main emission sources of the greenhouse gases. Manure based methane contributes 4% of all anthropogenic methane sources (Kafle and Chen, 2016). Again, large volume of gases, organic material, bacteria and other substances generated during livestock activities poses a risk factor in air, soil and water pollution (Mathias, 2014).

Anaerobic digestion offers an opportunity to deal with the problems of organic waste management, while diminishing environmental impact and providing a sustainable development of energy supply (Shah *et al.,* 2015; Ndubuisi-Nnaji *et al*., 2021). During anaerobic digestion, organic compound-degrading bacteria in the feedstock convert carbohydrate, proteins, polysaccharides and lipids into methane (CH_4) and carbon dioxide (CO_2). Biomethanation is a veritable process that treats organic wastes including livestock manure in the absence of oxygen, concurrently producing biogas and a digested residue (Ndubuisi-Nnaji *et al*., 2020b; Ofon *et al*., 2021). Biogas generated can be used as source of renewable energy. The digestate which is rich in macro- and micro-nutrients can be applied to soil to improve its fertility status or used as biofertilizer. However, the accurate application of anaerobic digestate on arable soil largely depend on the number and type of bacteria present in such digestate. The digestate must have low pathogen content to avoid the risk of transmission of pathogens between animals and humans (Coelho, 2018; Mortola *et al.,* 2019).

More so, the application of digestates and their impact on the environment and human health are mostly unexplored. Some articles have reported the agricultural potential and conflicting results of digestate performance (Nkoa, 2014, Coelho *et al*., 2018, Moller *et al.,* 2012). Along with the intensive development of animal husbandry, livestock manure production has increased dramatically. When untreated or not properly managed, livestock manure becomes a potential source of hazard to the environment and public health (Alburquerque *et al*., 2012b; Qi, 2018).

The existing body of research on anaerobic digestates has so far focused on pathogen survival and determination of their densities in digestate with scarce data about their susceptibility to commercial antibiotics (Ndubuisi-Nnaji *et al*., 2018; Resende *et al*., 2014; Manyi-Loh *et al*., 2014; Manyi-Loh *et al*., 2018; Coelho *et al*., 2018). Moreover, Nkoa (2014) reviewed extensively the impacts of soil applications of digestates on the broader environment. While current knowledge is grossly inadequate, a more robust exploration of the virulence determinants of bacteria recovered from digestates and their antibiotic susceptibility profile is lacking in published data and is worth investigating. The present study examined specifically the occurrence of virulence markers, hemolytic patterns and antibiotic susceptibility profiles of putative bacterial pathogens encountered during anaerobic digestion of manure before possible land application.

MATERIALS AND METHODS

Sample sources and Anaerobic Digestion Treatment

Fresh and untreated poultry manure was obtained from a commercial farm: Vika farms limited, located at Mbak Etoi, Uyo and goat manure samples was sourced from a loafing

shed located in a farm outbuilding (barn) in Ikot Ukot Anang, Ukanafun, L.G.A, both in Akwa Ibom State, Nigeria. The manure samples were collected into sterile containers and transported to Microbiology Laboratory of the University of Uyo, Uyo for further processing and anaerobic digestion treatment.

Two fabricated digesters operated under semi-batch digester system over a residence time of 45 days were used for the experiment. Slurry from both samples prepared (by mixing 1200g fresh manure with 1500 ml of water) after collection were fed separately into the lab-scale anaerobic digesters. Samples (100 ml) were collected through the outlet valve at the termination of digestion for isolation and identification of pathogens of interest for a period of 45 days following daily biogas production measurement via downward liquid displacement technique. The digesters were maintained under mesophilic temperature (28 ± 2ºC).

Isolation and characterization of indicator bacterial pathogens in digestate

Bacterial species were isolated by viable plate method. Ten (10) g of each wet slurry (sample) was serially diluted in 90ml of sterile distilled water and vigorously shaken to dislodge adhered bacterial cells. Ten-fold serial dilutions were made to obtain dilution of 10^{-1} and 10^{-3}. Using spread plate procedure, 0.1ml of aliquot was spread onto the surface of sterile agar plates in duplicate (Manyi-Loh *et al*., 2018). All media used were products of Sigma Aldrich, USA and they included Nutrient Agar (NA), MacConkey Agar (MCA), Eosin Methylene Blue (EMB) agar, *Salmonella Shigella* (SS) agar, Mannitol Salt agar (MSA), Thiosulphate Citrate Bile Salt agar (TCBS), Reinforced Clostridia agar (RCA). The NA, MCA, EMB, SSA, MSA, TCBS plates were incubated aerobically at 37 °C for 24 hours for aerobes and RCA plates was anaerobically incubated for 24 hours at 37 °C using the gaspak system for anaerobes. Discrete colonies on primary plates were sub-cultured onto freshly prepared agar plates and aerobically incubated overnight at 37 °C. The pure cultures of isolates were sub-cultured and stored on agar slants, in a refrigerator at 4 °C for further characterization and identification using appropriate biochemical test; indole, methyl red, Voges Proskauer, catalase, coagulase, oxidase and carbohydrate fermentation (Holt *et al*., 1994).

Determination of virulence attributes of bacterial isolates

The attributes assayed for included production of lecithinase (phospholipase), gelatinase, caseinase, amylase, and haemolysin following the methods of Akinjogunla *et al*. (2016), Pasquale *et al*. (2012), James and Natalie (2008), and Ndubuisi-Nnaji *et al*. (2018). For lecithinase production, plates of egg yolk agar were inoculated by streaking with bacterial isolates and incubated anaerobically at 37˚C for 72 h. The bacterial isolates were streaked on plates of gelatin agar to assay for gelatinase production and on skimmed milk agar plates for caseinase production, the plates were incubated aerobically at 37˚C for 48 h**.** Transparent, opaque or clear zones around the bacterial colonies indicated positive results. For amylase production, the isolates were streaked on plates of starch agar and incubated for 48 h at 37 °C. After incubation, 3 drops of 10 % Lugols iodine was added to the culture plates and allowed to react for 10 min. Clear zones around bacterial colonies indicated amylase production. The haemolysin production by the isolates was identified by the presence of haemolytic zones (clear – β or greenish halos - α, and no zones - γ) around the colonies on blood agar after incubation for 24 h at 37˚C.

Antibiotic susceptibility profile of bacterial isolates

In-vitro antibiotic susceptibility of bacterial isolates was determined using Kirby-Bauer disc diffusion technique (CLSI, 2016).

Briefly, 10 µL of each bacterial isolate, prepared directly from a 16-hr old agar plate and adjusted to 0.5 McFarland Standard, was inoculated on each plate of Mueller Hilton Agar (MHA). The antibiotic discs tested on Gram positive bacterial isolates were: Ciprofloxacin (CPX, 10 µg), Norfloxacin (NB,10µg), Gentamycin (CN, 10µg), Amoxicillin (AML, 20 µg), Streptomycin (S, 30 µg), Erythromycin (E, 30 µg), Ampicloxacillin (APX, 20 µg), Chloramphenicol (CH, 30 µg), Levofloxacin (LEV, 10 µg) and Rifampin (RD, 20 µg), while Ciprofloxacin (CPX, 10 µg), Pefloxacin (PEF, 10 µg), Augmentin (AUG, 30 µg), Cephalothin (CEP, 10 µg), Streptomycin (S, 30µg), Nalidixic Acid (NA, 30 µg), Ofloxacin (OFX, 10 µg), Trimethoprim-Sulphamethoxazole (SXT,30 µg) and Ampicillin (PN, 30 µg) were used for Gram negative bacterial isolates. The antibiotic discs were aseptically placed on the surfaces of the culture plates with sterile forceps, and the plates were incubated at 37 °C for 18 h. (Okon *et al*., 2020). Inhibitory zones observed were measured in millimeters (mm) and interpreted as sensitive, intermediate or resistant based on the criteria outlined in CLSI (2016).

RESULTS

A total of 75 bacterial isolates were characterized and identified from both poultry and goat manure samples. The isolates belonging to 10 genera consisted of 40 (53.3 %) gram-positive bacteria (GPB) and 35 (46.7 %) gram-negative bacteria (GNB). Of the 75 bacterial isolates, *Clostridium* sp, *B. subtilis* and *E. coli* had the highest frequency of occurrence (13.3 % each), followed by *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* 10.6% each, *Staphylococcus aureus* 9.33 %, *Vibrio cholerae and Salmonella* sp 8.00 % each, while least frequency of occurrence was recorded for *E. faecium* and *Shigella* sp 6.66% each. (Fig 1)

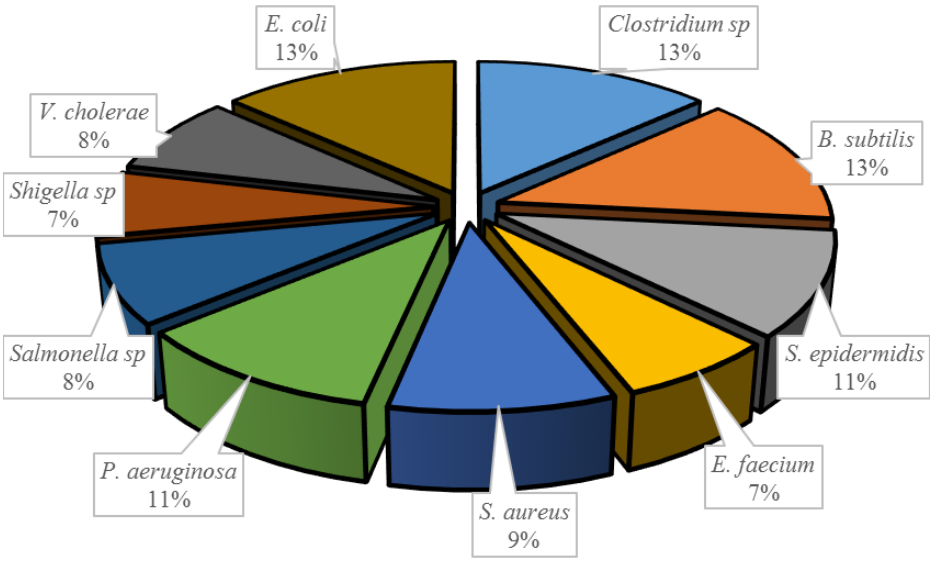

Figure 1: Percentage occurrence of bacterial isolates found in anaerobic digester

The virulence determinants and haemolysin patterns as seen in Tables 1 and 2 revealed that approximately 55 % of the isolates produced lecithinase, 56 % produced gelatinase, 43.0 % caseinase and 52.0 % amylases. These attributes were observed in more than 70% of *B. subtilis* strains and only in about 10 % of *E. coli* strains. The haemolysin production were in the order 35.0 %, 29 % and 36.0 % for alpha (α), beta (β) and gamma (γ) haemolysin, respectively.

Table 1: Virulence determinants of bacterial isolates

Bacterial Isolates	Total No. Tested	Virulence Factors							
		Lecithinase Production (%)		Gelatinase Production (%)		Caseinase Production (%)		Amylase Production (%)	
		(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)
Clostridium sp	10	2(20.0)	8(80.0)	9(90.0)	1(10.0)	7(70.0)	3(30.0)	2(20.0)	8(80.0)
B. subtilis	10	9(90.0)	1(10.0)	8(80.0)	2(20.0)	8(80.0)	2(20.0)	7(70.0)	3(30.0)
S. epidermidis	8	6(75.0)	2(25.0)	1(12.5)	7(87.5)	2(25.0)	6(75.0)	7(87.5)	1(12.5)
E. faecium	5	1(20.0)	4(80.0)	3(60.0)	2(40.0)	1(20.0)	4(80.0)	4(80.0)	1(20.0)
S. aureus	7	6(85.71)	1(14.29)	5(71.4)	2(28.6)	1(14.29)	6(85.71)	5(71.4)	2(28.6)
P. aeruginosa	8	2(25.0)	6(75.0)	7(87.5)	1(12.5)	6(75.0)	2(25.0)	6(75.0)	2(25.0)
Salmonella sp	6	5(83.3)	1(16.7)	2(33.3)	4(66.7)	1(16.7)	5(83.3)	5(83.3)	1(16.7)
Shigella sp	5	4(80.0)	1(20.0)	2(40.0)	3(60.0)	3(60.0)	2(40.0)	1(20.0)	4(80.0)
V. cholerae	6	5(83.3)	1(16.7)	4(66.7)	2(33.3)	2(33.3)	4(66.7)	1(16.7)	5(83.3)
E. coli	10	1(10.0)	9(90.0)	1(10.0)	9(90.0)	1(10.0)	9(90.0)	1(10.0)	9(90.0)
TOTAL	75	41(54.6)	34(45.3)	42(56.0)	33(44.0)	32(42.6)	43(57.3)	39(52.0)	36(48.0)

Key: + = Positive; - = Negative

Table 2: Haemolytic patterns of bacterial isolates in anaerobic digestate

Bacterial Isolates	HAEMOLYTIC PATTERNS OF ISOLATES (%)			
	Total number tested	Alpha (α)	Beta (β)	Gamma (γ)
Clostridium sp	10	2(20.0)	1(10.0)	7(70.0)
B. subtilis	10	6(60.0)	0	4(40.0)
S. epidermidis	8	1(12.5)	0	7(87.5)
E. faecium	5	1(20.0)	4(80.0)	0
S. aureus	7	3(42.8)	4(57.1)	0
P. aeruginosa	8	1(12.5)	0	7(87.5)
Salmonella sp	6	4(66.6)	0	2(33.3)
Shigella sp	5	1(20.0)	4(80.0)	0
V. cholerae	6	4(66.6)	2(33.3)	0
E. coli	10	3(30.0)	7(70.0)	0
TOTAL	75	26(35.0)	22(29.3)	27(36.0)

The antibiotic susceptibility patterns of Gram Positive Bacteria (GPB) and Gram Negative Bacteria (GNB) isolated from digesters treating animal manure is presented in Tables 3 and 4 respectively. The result of antibiotic susceptibility pattern showed that all (100 %) of the GPB were sensitive to ciprofloxacin and rifampicin. *Bacillus subtilis* and *Clostridium* sp were generally sensitive to all the antibiotics; with only 20 % of *Bacillus subtilis* resistance to norfloxacin,streptomycin and ampicloxacillin, and 20 % *Clostridium* sp. showed resistance to erythromycin and norfloxacin. *S. aureus* displayed 57.1 % resistance to both am oxicillin and levofloxacin (Table 3).

The GNB (Table 4) exhibited 100% sensitivity to ofloxacin, pefloxacin and ciprofloxacin, while, *E. coli* showed greater than 60 % resistance to ampicillin, trimethoprim-sulphamethoxazole and cephalothin. *P. aeruginosa* and *Vibrio cholerae* displayed 87 % and 67.7 % resistance to ampicill in and nalidixic acid, respectively.

Table 3: Antibiotic susceptibility testing of gram positive bacteria (GPB) isolated during AD of manure

		Antibiotic Sensitivity Profile No (%)									
Bacterial isolates	**Total No. Tested**	**CPX**	**NB**	**CN**	**AMX**	**S**	**RD**	**E**	**CH**	**APX**	**LEV**
S. aureus	7	7(100)	4(57.1)	6(85.7)	3(42.9)	6(85.7)	7(100)	6(85.7)	6(85.7)	5(71.4)	3(42.9)
E. faecium	5	5(100)	4(80)	5(100)	4(80)	5(100)	5(100)	5(100)	5(100)	4(80)	4(80)
S. epidermidis	8	8(100)	7(87.5)	8(100)	5(62.5)	8(100)	8(100)	8(100)	8(100)	8(100)	7(87.5)
B. subtilis	10	10(100)	10(100)	10(100)	10(100)	8(80)	10(100)	10(100)	10(100)	8(80)	10(100)
Clostridium sp	10	10(100)	8(80)	10(100)	10(100)	10(100)	10(100)	8(80)	10(100)	10(100)	10(100)
Total	40	40(100)	33(82.5)	39(97.5)	32(80)	37(92.5)	40(100)	37(92.5)	39(97.5)	35(87.5)	34(85)

Key: CPX= Ciprofloxacin, NB= Norfloxacin, CN= Gentamycin, AMX= Amoxicillin, S= Streptomycin, RD= Rifampicin, E= Erythromycin, CH= Chloramphenicol, APX= Ampicloxacillin, LEV= Levofloxacin

Table 4: Antibiotic susceptibility profile of gram negative bacteria (GNB) isolated during AD of manure

		Antibiotic Sensitivity Profile No (%)									
Bacterial Isolates	**Total No. Tested**	**OFX**	**PEF**	**CPX**	**AU**	**CN**	**S**	**CEP**	**NA**	**SXT**	**PN**
P. aeruginosa	8	8(100)	8(100)	8(100)	4(50)	7(87.5)	8(100)	3(37.5)	5(62.5)	6(75)	1(12.5)
E. coli	10	10(100)	10(100)	10(100)	6(60)	8(80)	10(100)	4(40)	7(70)	3(30)	3(30)
V. cholerae	6	6(100)	6(100)	6(100)	3(50)	6(100)	5(83.3)	6(100)	2(33.3)	5(83.3)	3(50)
Shigella sp.	5	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)	4(80)
Salmonella sp	6	6(100)	6(100)	6(100)	4(66.6)	4(66.6)	6(100)	4(66.6)	5(83.3)	4(66.6)	4(66.6)
Total	35	35(100)	35(100)	35(100)	22(63.0)	30(85.7)	34(97.1)	22(62.9)	24(68.6)	23(65.7)	15(43)

Key: OFX = Ofloxacin, PEF = Pefloxacin, CPX = Ciprofloxacin, AU = Augmentin, CN = Gentamycin, S= Streptomycin, CEP= Cephalothin, NA= nalidixic acid, SXT= Trimethoprim-Sulphamethoxazole, PN = Ampicilin

DISCUSSION

Microorganisms such as *Escherichia coli, Staphylococcus aureus, Vibrio cholerae, Enterococcus faecium, Staphylococcus epidermidis, Salmonella* spp, *Shigella* spp, *Bacillus subtilis, Clostridium* spp, *Pseudomonas aeruginosa* were isolated, characterized from digesters treating animal manure. Some of these organisms isolated were members of the gastrointestinal tracts of warm blooded animals and when shed via faeces can pollute the environment resulting in public health related issues (Manyi-Loh *et al*., 2018).

Of the 75 bacterial isolates encountered, those that exhibited virulence factors were approximately: 55% for lecithinase, 56 % for gelatinase, 43% for caseinase and 52% for amylases while haemolysin production were 35%, 29% and 36% for alpha, beta and gamma haemolysis, respectively. Both virulence attributes and haemolysin production were mostly observed among the gram positive bacteria (GPB). The presence and elaboration of virulence markers like lecithinase, gelatinase, caseinase, amylase and haemolysin among encountered organisms confirmed that the digestate harboured pathogenic strains of organisms. In a similar study by Ndubuisi-Nnaji *et al*. (2018), virulence determinants were not recorded among bacteria recovered from anaerobic digesters treating bio-waste. However, they reported the production of haemolysin among bacterial species in anaerobic digesters. The disparity in the isolates display of virulence determinants signified their varying degree of pathogenicity (Umana *et al*., 2017). The variation in virulence profiles of organisms may be attributed to the difference in sample source. The isolation of amylase producing *Bacillus* sp agrees with previous findings of Oyeleke *et al*. (2011). Amylase is a key hydrolytic enzyme for the degradation of starch, a component of living tissues. Haemolysin patterns of *Staphylococcus epidermis, Clostridium sp* and *P. aeruginosa* from our findings corroborate the report by Ndubuisi-Nnaji *et al*. (2018) that high gamma-haemolysin production from *Staphylococcus epidermis, Clostridium sp* and *P. aeruginosa* isolated from feedstock and digestate samples which was closely followed by alpha haemolysin; while majority of *E. faecium* produced more beta haemolysin. The pathogenicity of *Staphylococcus* is attributable to its tendency to elicit virulence factors such as coagulase and haemolysin and their occurrence in this study conforms to the report of Nester *et al*. (1998).

Humans have been reported to be adversely affected by antimicrobial resistance strains of some gram-negative bacteria particularly *E. coli*, *Salmonella* sp, *Vibrio cholerae* and *Shigella* sp obtained from livestock wastes due to their constant interactions with agrowastes (Manyi-Loh *et al*., 2018; Shanks and Peteroy-Kelly, 2009). There is a great tendency for antibiotic-resistant bacteria with resistance genes to be disseminated via air, water, food, and rainfall; into environment being their final reservoir (Abo-State *et al*., 2012). The bacterial isolates displayed varying levels of susceptibility to the tested antibiotics. Generally, the gram positive bacteria (GPB) exhibited approximately 100 % sensitivity to most antibiotics and 17.5 % and 20 % resistance to norfloxacin and amoxicillin, respectively; while the gram negative bacteria (GNB) displayed 38.1 % and 57 % resistance to cephalothin, ampicillin, respectively and approximately 100% sensitivity to other antibiotics. The GPB displayed varied level of 100 % resistance to a single antibiotic whereas members of the family Enterobacteriaceae displayed 100 % sensitivity to about 3 to 4 antibiotics of the tested antibiotics which was inconsistent with the works of Manyi-Loh *et al*. (2018) who characterized antibiotic resistance of selected bacterial pathogens recovered from dairy cattle manure during anaerobic mono-digestion in a balloon-type digester. Altogether, the most effective antibiotics was ciprofloxacin.

Although, different bacterial isolates showed varied levels of resistance to a single antibiotic, some were resistant to more than one antibiotics for example: 57.1 % resistance of *S. aureus* to amoxicillin and levofloxacin, respectively; 87.5 % resistance of *P. aeruginosa* to ampicillin and 66.7 % of *V. cholerae* to naldixic acid, while, *E. coli* exhibited 70 % resistance to Trimethoprim-Sulphamethoxazole (SXT) and Ampicillin (PN). These observations corroborated previous studies by Abo-State *et al*. (2012), Manyi-Loh *et al*. (2018) and Simango, (2013) that separately investigated the antibiotic resistance of enteropathogens from environmental media. The varying degrees of antibiotic susceptibility among the isolates could be linked to the different antibiotic pressures these organisms are posed to in manure waste as well as its origin. The implication of these findings remains that digestate, when used as fertilizer can present a potential source for and spread of antibiotic resistance, hence the need for continuous post-digestion surveillance and sanitation.

CONCLUSION

Anaerobic digestates from animal (goat and poultry) manure harboured putative pathogens with virulence attributes and antibiotic resistant traits, signifying their potential to contaminate the environment and subsequent infect plants, animals and humans. Data from this study would serve as a baseline for future research(es) on search for virulence markers responsible for pathogenicity and antibiotic resistance in anaerobic digestates. Post anaerobic hygienization or digestion treatment (PAHDT) of digestate should be encouraged to produce a hygienically safe digestate that meets regulatory standards. Biosafety risk assessment is strongly recommended before land application of digestate.

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