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# Antimicrobial effects of plant extracts and essential oils on multi resistant Enterobacteriaceae isolated from animal waste samples.

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**Abstract**
The need to continuously search for alternative antimicrobial chemotherapy is necessitated by the ever increasing resistance to antimicrobials amongst microbial pathogens. This study aimed at evaluating the effect of plant extracts and essential oils on antimicrobial resistant enterobacteriaceae isolated from poultry and piggery environments. Four hundred isolates of *Klebsiella pneumoniae* and *Escherichia coli* obtained from the two environmental sources were tested for their susceptibility to ten (10) antimicrobials by the Kirby-Bauer technique. The isolates were also tested for extended β-lactamase production by the modified double disc synergy test and the effects of essential oils and extracts from *Moringa oleifera* seeds and *Ocimum gratissimum* (scent leaf) leaves on the isolates were analyzed using the agar in well diffusion assay. The *E. coli* isolates expressed high rates of resistance for the following antibiotics: ampicillin (93%), cefotaxime (78%) ceftazidime (57%) and moderate rates for imipenem (54.5%), aztreonam (50%), while *K. pneumonia* isolates expressed high resistance rates for the following antimicrobials respectively: ampicillin (98.5%), cefotaxime (97%) and ceftazidime (88.5%). *E. coli* generally expressed higher resistance rates than the *K. pneumonia* isolates. Thirty-seven (59%) of sixty-five isolates tested were found to be positive for ESBL production. Antimicrobial analysis of the essential oils against the ESBL producers showed no inhibitory activity while the plant extracts produced zones of inhibition and minimum inhibitory concentrations that ranged from 1.32mg/ml to 1.67mg/ml for *Klebsiella pneumoniae* and 1.32mg/ml for *E. coli* for the two tested plant extracts. Phytochemical analysis showed the presence of alkaloids, tannins, saponins, flavonoids and glycosides. *Moringa oleifera* seed and *Ocimum gratissimum* leaf crude extracts could be used as effective plant extracts for the eradication of antimicrobial resistant enterobacteriaceae.

**Keywords**: Antimicrobials, microbial pathogens, *Moringa oleifera,* multi-resistant, *Ocimum gratissimum*, plant extracts

## 1. Introduction

The indiscriminate use of antibiotics in livestock and animal husbandry had led to alarming increases in antimicrobial resistance in associated bacteria (Shivakumaraswamy *et al.,* 2019) including members of the enterobacteriaceae. This therefore brings to the fore the

need and importance to continuously monitor the physical and agricultural environments as important reservoirs of multidrug resistant bacteria of public health importance. According to Xi *et al.* (2009), it is easy for organisms isolated from environments with high faecal contamination to acquire resistance to common antimicrobial drugs. Also, the prophylactic use of antibiotics in agricultural settings including piggeries and poultries has added to increased incidence of multiple drug resistant organisms in those environments (Collignon *et al.,* 2005; Dibner *et al.,* 2005).

Antimicrobial resistance has become a major health problem that is rapidly spreading across the world and has been brought on by the abuse and misuse of antibiotics in both food animals and medical practice. Antimicrobial resistance can spread from animals to humans and vice versa, either directly by the transfer of the resistant bacteria or indirectly by the spread of the resistant genes from animal bacteria to bacteria found in humans (Dibner *et al.,* 2005; Sarter *et al..,* 2007).This has also increased the risks of untreatable or difficult to treat infections (Argudin *et al.,* 2017). Unfortunately, according to Shivakumaraswamy *et al.* (2019), the emergence of and increases in antimicrobial resistance has been accompanied with a reduction in the discovery of new agents. Continuing, he opined that most of the antibiotics currently in use for common human and animal infections would have outlived their usefulness in another 5 to 10 years.

The need to monitor and understand antibiotic resistance patterns of human bacterial pathogens that persist in the environment cannot be overemphasized (Shivakumaraswamy *et al.,* 2019), if we are to maintain the sanctity of clinical medicine and chemotherapy. This has therefore called for a concerted effort in the search for alternative antimicrobials chemotherapies being that most pathogens have developed resistance to commonly used commercial antimicrobials.

The enterobacteriacea are a common cause of nosocomial infections and belong to the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter pneumoniae, Pseudomonas aeruginosa* and *Enterobacter*) group of organisms. These organisms comprise highly-multi-extended or pan-drug resistant strains that cause a large number of both clinical and community infections with limited treatment options. Currently the searchlight is beamed on them to monitor their role on the maintenance of antimicrobial resistance and dissemination of resistance genes (Argudin *et al*., 2017). According to Shobar and Abo-Amer (2014), the gut of poultry birds can be a reservoir of *E. coli,* which can further be transmitted to human by contact with the poultry wastes or contaminated poultry foodstuffs (Aly and Abo-Amer, 2018).
To mitigate against the poor response of antibiotics to resistant organisms (both pathogenic and commensals), it is necessary to continuously search for new antimicrobials that could be used as effective treatment against multi-drug resistant organisms which are also capable of transmitting resistance markers to other bacteria in their environment. Herbal Plant extracts and essential oils have been studied over the years as potential sources of new antimicrobials that can be applied in antimicrobial chemotherapy. This work was therefore carried out with the aim of assessing antimicrobial resistance in *Escherichia. Coli* and *Klebsiella pneumoniae* isolated from piggery and poultry environments as well as the antimicrobial effects of essential oils and plant extracts against these resistant bacterial pathogens.

## 2. Materials and Methods

### 2.1. Sample types and collection

Four animal waste types were used in the study: 20 chicken cloacal swabs and 20 chicken waste samples, 30 pig rectal swabs and 20 pig waste samples. The chicken cloacal and pig rectal swab samples were collected with sterile swab sticks, dipped into the cloacal and anal regions of the chickens and pigs respectively and rotated round to collect enough sample materials. The faecal waste samples were collected using sterile spatula and put into 20ml sterile plastic sampling bottles (Ejikeugwu *et al*., 2017; Amaechi and Nwankwo, 2015).

*Moringa oleifera* seeds and *Ocimum gratissimum* leaves were obtained from the Relief market in Owerri Municipal Council Area, Imo State, Nigeria. The plant samples were confirmed by a plant taxonomist at the Crop Science Technology department of the Federal University of Technology, Owerri Imo State, Nigeria. Ten antimicrobials were analyzed for activity against the isolates: gentamycin (10μg), imipenem (10μg), ampicillin (10μg), levofloxacin (5μg), ceftazidime (30μg), aztreonam (30μg), cefotaxime (30μg), ciprofloxacin (5μg), meropenem (10μg) and ertapenem (10μg) (Oxoid, UK). The breakpoints in μg/ml for resistant isolates according to Clinical and Laboratory Standards Institutes (CLSI) (2019) for the antimicrobials are as follows: gentamycin ≤12, ertapenem≤ 19, imipenem≤ 19, meropenem≤ 19, ciprofloxacin ≤ 15, levofloxacin ≤ 13, ceftazidime≤ 17, aztreonam≤ 17, cefotaxime≤ 22, ampicillin ≤ 13.

### 2.2. Isolation, purification and characterization of bacterial isolates

Primary isolation of *Escherichia coli* and *Klebsiella pneumoniae* was carried out using MacConkey and Eosin Methylene Blue Agar, after prior enrichment in nutrient broth at 30$^{o}$C for 24 hours. At the end of incubation, 2 or 3 distinct characteristic colonies were selected for further purification into pure colonies and subsequent characterization and confirmation using the IMViC tests for enterobacteriaceae (Cheesbrough, 2004)

### 2.3. Antibiotic resistance testing and phenotypic tests for extended spectrum beta-lactamase production

Antibiotic resistant analysis of the isolates was conducted by the Kirby-Bauer technique as described by Cheesbrough (2004). A loop-full of isolates standardized to 0.5 McFarland standards was spread on the dried surface of Mueller-Hinton agar plates using sterile swab sticks. Antimicrobial discs were then placed on the agar surface using sterile forceps, the plates left briefly on the table to dry and were subsequently incubated at 35$^{o}$C for 24 hours. Zones of clearing were measured with a meter rule and noted as either resistant or sensitive according to the established breakpoints.

The phenotypic testing for ESβL producing isolates was conducted with isolates that showed resistance to ceftazidime and cefotaxime by the modified double disk synergy test (MDDST) according to Kaur *et al*. (2013) using third generation cephalosporins; cefotaxime, ceftriazone and cefpodoxime, and one fourth generation cephalosporin (4GC), cefepime, with amoxicillin-clavulanate (20/10μg) combination disc. The standardized isolates were spread on Mueller-Hinton agar plates, and discs of amoxicillin-clavulanate placed in the center while the other antibiotics were placed 20mm center to center to that of the combination disc at the center. An increase or distortion in the zones of inhibition towards the combination disc was considered as positive for ESβL production.

### 2.4. Extraction of plant samples

For the crude ethanol extraction of plant samples, the dried powdered leaves and seeds were soaked in ethanol for four days, with constant shaking. The mixture was subsequently filtered using Whatman No 1 filter paper and the centrifuged at 1500xg for 20 minutes. The crude extract was evaporated to dryness by placing in a water bath at 40$^{o}$C and then put in capped containers and stored at 4$^{o}$C as plant stock. For the extraction of essential oil from the leaf and seed samples, the technique of Adepoju *et al.* (2014) was adopted. Soxhlet extractor of capacity 250ml was used, with petroleum ether as solvent and 50 g fresh plant materials. The quantity of the oil extracted was ascertained gravimetrically as the ratio of the weight of the extracted oil to the weight of the plant materials used. The extracted oils were kept in the refrigerator at 4$^{o}$C until required for the analysis.

### 2.5. Antimicrobial effects of the essential oils and plant extracts against ESβL isolates

Antimicrobial effects of the essential oils were determined by the disc method of Şerban *et al.* (2011) and agar diffusion methods of Donaldson *et al.* (2005). Paper discs, prepared from Whatman No 1 filter paper, using a puncher were sterilized for 180 minutes at 170$^{o}$C. Different concentrations of the *Moringa oleifera* and *Ocimum gratissimum* essential oils were now dropped on the discs for analysis, after dissolving different quantities of the oil in appropriate volumes of dimethyl sulfoxide (DMSO). The following concentrations were used for the assay: 10µl (1ml of essential oil), 7.5µl (0.75 ml of essential oil and 0.25 ml of DMSO), 5µl (0.5ml of essential oil and 0.5ml of DMSO), and 2.5µl (0.25mls of essential oil and 0.75ml of DMSO). Each disc had a capacity of 0.01ml of the solution. Assay for the activity of both essential oils and crude ethanol plant extracts were carried out by the well-diffusion assay (Donaldson *et al*., 2005). For the essential oils, 0.2ml of the standardized inocula of the test organisms were spread on Mueller-Hinton agar plates in duplicates. Wells were dug on the plates using 6mm diameter cork borer and filled with 10, 20 and 30 µl of the essential oils. The plates were subsequently sealed with paraffin to avoid evaporation of the essential oils, allowed for 30minutes at room temperature for the oils to diffuse through and then incubated at 37$^{o}$C for 24 hours.

For the assay of the crude ethanol extracts (CEE) of the test plant materials, standardized inocula were also swabbed on the Mueller-Hinton agar, allowed to dry before holes of 6mm in diameter were made on the seeded agar surface using a sterile cork borer. Aliquots of 100mg/ml, 25mg/ml and 12.5mg/ml of the CEE were added into each well and allowed to stand for proper diffusion before incubating for 24hours at 37$^{o}$C. Diameters of the inhibition zones were measured in millimeters after incubation (Perez *et al*., 1990).

## 3. Results

A total of 200 each of *Escherichia coli* and *Klebsiella pneumoniae* were isolated from the piggery and chicken environments. *E.coli* expressed high resistance rates for ampicillin (93%) and cefotaxime (78%), while resistance rates were moderate for ceftazidime (57%), imipenem (54.5%), aztreonam (50%), ertapenem (42.5%), ciproflaxacine (42%) and low for meropenem (38%), and levofloxacine and gentamycin (25.5% each). The chicken waste isolates also had higher resistance rates to the antimicrobials tested than isolates from all the other samples (Table 1). The *Klebsiella pneumoniae* isolates expressed high resistance rates for ampicillin (98.5%), cefotaxime (97%), ceftazidime (88.5%), ertapenem (83.5%), imipenem (83%), meropenem (82.5%), aztreonam (80.5%), and moderate for ciprofloxacine

(57%). Levofloxacine (45.5%) and low for gentamycin (38.5%). The highest rates of resistance were also recorded for isolates from chicken waste samples (Table 2). Comparatively, *K. pneumoniae* isolates expressed higher resistance rates for the same antibiotics then the *E. coli* isolates as shown on Table 3.

A total of 65 resistant isolates from both environmental samples were analyzed for phenotypic production of ESβL, out of which 37 (56.9%) were identified as ESβL producers. All 37 isolates showed a distinct extension of the edge of the inhibition zones produced by the carbapenems towards the amoxicillin – clavulanate combination disc. Of these 21 (32.3%) were *E. coli* while 16 (24.6%) were *K. pneumoniae* isolates (Table 4).

The isolates expressed varying numbers of resistance patterns, with *E.coli* from pig rectal swabs exhibiting 40 patterns and the least being *K. pneumoniae* isolates from pig rectal swab exhibiting 12 patterns (Table 5). The multiple antibiotic resistance (MAR) index fell between 0.3 and 1 with up to 90% of the isolates from the different environment sources being resistant to between 3 and all 10 antimicrobials tested. In all 16 isolates were resistant to only one antibiotic.

There was inhibition of the bacterial isolates at 100mg/ml, 50mg/ml and 25mg/ml by both the CAEs and CEEs of the plant materials. Also minimum inhibition concentrations of between 1.32mg/ml and 1.67mg/ml were recorded for the two plant extracts as seen on Tables 6 and 7. However, the *M. oleifera* seed and *O. gratissimum* leaf essential oils were not active against the ESβL producing isolates for both the disc diffusion and agar-well diffusion protocols, since no zones of inhibition were noticed.

Quantitative and qualitative phytochemical analysis of the extracts showed the presence of alkaloids, saponins, tannins, flavonoids, steroids, glycosides and terpenoids in varying amounts as shown on Table 8.

Table 1: Antimicrobial resistance profile of E*scherichia coli* isolated from chicken and pig samples

| Antibiotics | Sample type/resistance rates (%) Pig rectal swab (n=50) | Pig waste (n=50) | Chicken waste (n=50) | Chicken cloacal swab (n=50) | Total (n=200) |
|---|---|---|---|---|---|
| Ampicillin | 47 (94) | 46 (92) | 50 (100) | 43 (86) | 186 (93) |
| Cefotaxime | 42 (84) | 44 (88) | 46 (92) | 24 (48) | 156 (78) |
| Ceftazidime | 19 (38) | 29 (58) | 46 (92) | 20 (40) | 114 (57) |
| Imipenem | 27 (54) | 22 (44) | 49 (98) | 11 (22) | 109 (54.5) |
| Aztreonam | 26 (52) | 22 (44) | 47 (94) | 5 (10) | 100 (50) |
| Ertapenem | 30 (60) | 16 (32) | 28 (56) | 11 (22) | 85 (42.5) |
| Ciprofloxacin | 15 (30) | 10 (20) | 41 (82) | 18 (36) | 84 (42) |
| Meropenem | 14 (28) | 17 (34) | 39 (78) | 6 (12) | 76 (38) |
| Levofloxacin | 6 (12) | 7 (14) | 32 (64) | 6 (12) | 51 (25.5) |
| Gentamycin | 9 (18) | 6 (12) | 27 (54) | 9 (18) | 51 (25.5) |

Table 2: Antimicrobial resistance profile of *Klebsiella pneumoniae* isolated from chicken and pig samples

| Antibiotics | Sample type / resistance rates (%) Pig rectal swab (n=50) | Pig waste (n=50) | Chicken waste (n=50) | Chicken cloacal swab (n=50) | Total (n=200) |
|---|---|---|---|---|---|
| Ampicillin | 49 (98) | 49 (98) | 50 (100) | 49 (98) | 197 (98.5) |
| Cefotaxime | 49 (98) | 50 (100) | 48 (96) | 47 (94) | 194 (97) |
| Ceftazidime | 44 (88) | 50 (100) | 47 (94) | 36 (72) | 177 (88.5) |
| Ertapenem | 44 (88) | 49 (98) | 48 (96) | 26 (52) | 167 (83.5) |
| Imipenem | 48 (96) | 37 (74) | 43 (86) | 38 (76) | 166 (83) |
| Meropenem | 44 (88) | 38 (76) | 43 (86) | 40 (60) | 165 (82.5) |
| Aztreonam | 50 (100) | 45 (90) | 26 (52) | 40 (80) | 161 (80.5) |
| Ciprofloxacin | 24 (48) | 19 (38) | 37 (74) | 34 (68) | 114 (57) |
| Levofloxacin | 20 (40) | 14 (28) | 39 (78) | 18 (36) | 91 (45.5) |
| Gentamycin | 4 (8) | 11 (22) | 27 (54) | 35 (70) | 77 (38.5) |

Table 3: Comparative rates (%) of resistance between *E. coli* and *K. pneumonia*e isolates from chicken and pig samples

| Antibiotics | Isolates / resistance rates *E. coli* (n=200) | *K. pneumoniae* (n=200) |
|---|---|---|
| Ampicillin | 186 (93) | 197 (98.5) |
| Cefotaxime | 156 (78) | 194 (97) |
| Ceftazidime | 114 (57) | 177 (88.5) |
| Imipenem | 109 (54.5) | 166 (83) |
| Aztreonam | 100 (50) | 161 (80.5) |
| Ertapenem | 85 (42.5) | 167 (83.5) |
| Ciprofloxacin | 84 (42) | 114 (57) |
| Meropenem | 76 (38) | 165 (82.5) |
| Levofloxacin | 51 (25.5) | 91 (45.5) |
| Gentamycin | 51 (25.5) | 77 (38.5) |

Table 4: Number (%) of ESβL producing isolates amongst the chicken and piggery isolates

| Isolate | Sample type Chicken cloacal swab (n=16) | Chicken waste (n=16) | Pig rectal swab (n=13) | Pig waste (n=20) | Total (n=65) |
|---|---|---|---|---|---|
| *E. coli* | 5(31.3) | 6(37.5) | 2(15.4) | 8(40) | 21(32) |
| *K. pneumoniae* | 2(12.5) | 4(25) | 3(23) | 7(35) | 16(24.6) |

Table 5: Antimicrobial resistance patterns of the isolates

| Isolate | Source | No of resistance patterns | Predominant pattern | No of isolates expressing predominant pattern |
|---|---|---|---|---|
| *E. coli* | Pig rectal swab | 40 | CTX+IPM+AMP | 3 |
| | | | CTX+IPM+AMP+ATM | 3 |
| | Pig waste | 35 | CTX+AMP+ATM | 5 |
| | Chicken cloacal swab | 29 | AMP | 13 |
| | Chicken waste | 18 | CAZ+CTX+ETP+ATM+AMP+IMP+CIP | 15 |
| *K. pneumoniae* | Pig rectal swab | 12 | CAZ+CTX+ETP+ATM+AMP+IPM+CIP+MEM | 11 |
| | | | CAZ+CTX+ETP+ATM+AMP+IPM+MEM | 11 |
| | Pig waste | 19 | CAZ+CTX+ETP+ATM+IPM+MEM | 6 |
| | | | CAZ+CTX+ETP+ATM+AMP+IPM+CIP+MEM | 6 |
| | Chicken cloacal swab | 37 | CAZ+CTX+ETP+IPM+MEM+CIP+AMP+ATM | 7 |
| | Chicken waste | 22 | CAZ+CTX+ETP+LEV+ATM+AMP+IPM+CN+CIP +MEM | 19 |

**KEY:** CN (GENTAMYCIN), IMP (IMIPENEM), AMP (AMPICILLIN), LEV (LEVOFLOXACIN), CAZ (CEFTAZIDIME), ATM (AZTREONAM), CTX (CEFOTAXIME), CIP (CIPRPFLOXACIN), MEM (MEROPENEM), ETP (ERTAPENEM).

Table 6: Minimum inhibitory concentration (MIC) of the plant extracts against *K. pneumoniae*

| Test organism | Source | MIC (mg/ml) *M.oleifera* | O. gratissimum |
|---|---|---|---|
| *Klebsiella pneumoniae* | | | |
| | Pig waste Pig | 1.32 | indeterminate |
| | Rectal Swab | 1.67 | 1.32 |
| | Chicken Waste | 1.32 | 1.32 |
| | Chicken Cloacal Swab | 1.32 | 1.32 |

Table 7: Minimum inhibitory concentration (MIC) of the plant extracts against *E. coli*

| Test organism | Source | MIC (mg/ml) *M. oleifera* | O. gratissimum |
|---|---|---|---|
| *E.coli* | | | |
| | Pig waste Pig | 1.32 | 1.32 |
| | Rectal Swab | 1.32 | 1.32 |
| | Chicken Waste | 1.32 | 1.32 |
| | Chicken Cloacal Swab | 1.32 | 1.32 |

Table 8: Phytochemical constituents of the crude aqueous and crude ethanol extracts of the test plants

| | *O. gratissimum* | | *M. oleifera* | |
|---|---|---|---|---|
| | OE | OW | OE | OW |
| Alkaloids | + | - | +++ | ++ |
| Saponins | + | + | ++ | ++ |
| Tannins | + | + | ++ | + |
| Flavonoids | + | + | ++ | + |
| Steroids | - | - | | |
| Glycosides | + | + | + | ++ |
| Terpenoids | | | + | ++ |

KEY: OE; ethanol extract; OW: water extract; +; slightly present; ++; moderate: +++; abundant

## 4. Discussion

Resistance rates for antibiotics used in this study were high for common antibiotics like ampicillin, ceftazidime and cefotaxime for both *E. coli* from chicken and pig samples. This is in contrast to Abo-Amer *et al*. (2018) whose *E. coli* isolates from chicken expressed moderate resistance rates of 51% to ampicillin and low rates of 1% for ceftazidime and other beta-lactam antibiotics analyzed. Similar moderate rates were also observed for ciprofloxacine (59%), and low rates for levofloxacine (1%) and gentamicin (21%) like in the present study. However while rates for aztreonam were low for their study, it was moderate for the present study at 50%.

In a similar study by Duru *et al.* (2013) ESβL producing *E. coli* isolates from poultry samples in Owerri, Nigeria, were completely resistant to ampicillin, ceftazidime, cefotaxime, which is similar to the results of the present study. Their isolates were however completely susceptible to imipenem while resistance rates were moderate in the present study. The high rates of resistance recorded for ampicillin in this study is in agreement with studies from China which reported high resistance rates to ampicillin of 98% amongst enterobacteriaceae from chicken carcasses (Wu *et al*., 2016). In addition and consistent with the results of the present study, enterobacteriaceae from healthy chicken in Portugal expressed moderate resistant rates to flouroquinolone of 47% (Mendonça *et al*., 2016). Contrary to Amador *et al.* (2019), who detected no resistance to imipenem and meropenem, high rates of resistance (83%) for *K. pnuemoniae* and moderate rates (54.5%) for *E. coli* were identified amongst the isolates for imipenem in the present study and 38% and 82.5% for meropenem for *E. coli* and *K. pneumonia*e respectively. In a related study, and similar to the results of the present study, enterobactericeae isolated from river and aquaculture water samples expressed high resistant rates to ampicillin, cefotaxime, and imipenem (99%, 83% and 77%) for river water and 95% and 86% for ampicillin and ceftazidime for aquaculture water isolates (Chikwendu *et al.,* 2019). Resistance rates for *K. pneumoniae* were significantly higher than the *E. coli* isolates for the same antibiotics in the present study and this trend was also the same for river and aquaculture water isolates in Chikwendu *et al.* (2019).

The presence of antimicrobial resistant bacteria in livestock wastes and environment raises a lot of issues because of not only the promiscuous nature of bacteria to transfer from one environment to another, but also the ability of transfer of resistance markers to transfer from one bacterium to another and even to humans, especially after prolonged contact with other bacteria in the same environment. Indeed the livestock environment serves as a perfect one

for the transfer and dissemination of resistant pathogenic bacteria. These multi-drug resistant organisms can also acquire additional resistance markers (Karlowsky et al., 2003). Also according to Abo-Amer *et al.* (2018*),* antimicrobial resistant *E. coli* have been known to persist in the gut of poultry birds for long periods with or without antibiotic use, where they are able to serve as a route through which they are transferred to human population or even through consumption of the poultry meat.

The varied and many numbers of resistance patterns identified amongst the livestock isolates indicate a great level of variability among them, indicating the complex nature of the antimicrobial response of the organisms under study. This could be as a result of the different and varied types of antimicrobials applied in these farms for both prophylaxis and therapeutics which confer a selection pressure on the organisms. Multiple antibiotic resistance (MAR) index of 0.3 – 1 is an indication that the poultry and piggery environments are potential health risk environments since their MAR index values were above 0.25 (Krumperman, 1983; Hinton *et al.,*1985). Diverse environments in many different countries have been designated as potential health risk environments, attesting to the notion that the burden of MAR bacteria in the physical environment is a worldwide rather than a national or regional problem (Okeke and Edelman, 2001; Knapp *et al.,* 2012*;* Lupan *et al.,* 2017).

Even though the essential oils from the two test plants did not show any activity against the isolates, there was visible antimicrobial activity with crude aqueous and ethanol extracts of the plants with MICs ranging between 1.32 and 1.67mg/ml of the plant extracts. Many other plants have also been found active against resistant pathogenic microorganisms like *Vernonia amygdalina* and *Croton macrostachyus* against bacterial and fungal pathogens (Habtom and Gabrehiwot, 2019), *Allium sativum* L. (garlic), *Thyme vulgaris* L (thyme)*,*and *Allium capa* L. (onion) against *Aeromonas* spp isolated from farmed common Carp (Daood, 2011). Some phytochemicals were identified on analysis of the plant extracts, including saponins, alkaloids, terpenoids, glycosides, steroids, flavonoids and tannins. The antimicrobial activities of the plant extracts could be as a result of the presence of these phytochemicals.

## 5. Conclusion

Very high rates of resistance were found amongst the isolates in this study, as well as extended spectrum beta lactamase activity, with up to 90% of them expressing multiple antibiotic activities. However, with antimicrobial activity of the crude aqueous and ethanol extracts of *Moringa oleifera* and *Ocimum gratissimum* against the livestock isolates, these plants could be potential sources of new and effective alternative antimicrobials for the treatment of multiple resistant *E. coli* and *K. pneumoniae* in these environments. This will ultimately go a long way to control the spread and persistence antimicrobial resistance in both the clinical and physical environments.

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