Antimicrobial Resistance among Enterobacteriaceae Found in Chicken and Cow Droppings and Their Public Health Importance

Chukwuebuka Chisom Anene¹,², Angus Nnamdi Oli¹*, Peter Anyigor Edeh¹, Moses Ugochukwu Okezie¹, James-Paul Kretchy³

¹Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Agulu, Nnamdi Azikiwe University, Awka, Nigeria
²Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria
³Department of Physician Assistantship Studies, School of Medicine and Health Sciences, Central University, Accra, Ghana

Email: *a.n.oli@live.com

How to cite this paper: Anene, C.C., Oli, A.N., Edeh, P.A., Okezie, M.U. and Kretchy, J.-P. (2021) Antimicrobial Resistance among Enterobacteriaceae Found in Chicken and Cow Droppings and Their Public Health Importance. Advances in Microbiology, 11, 694-711. https://doi.org/10.4236/aim.2021.1111050

Received: October 17, 2021
Accepted: November 27, 2021
Published: November 30, 2021

Copyright © 2021 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Abstract

Introduction: The recent surge in the number of antimicrobial resistant cases from hospitals and communities has created a need to study the points and sources of exposure to certain bacteria and determine their susceptibility to commonly used antibiotics. This study aimed at identifying and screening for drug-resistant Enterobacteriaceae isolated from chicken droppings and cow dungs in Onitsha, Anambra state, in the South-Eastern part of Nigeria. Methods: This is a cross-sectional descriptive study which included 50 chickens and 50 cow dung samples collected from five poultry houses and cow ranches respectively using sterile swab sticks. The samples were transported to the laboratory and processed following standard microbiological protocols. Isolates in the samples were recovered using MacConkey Agar, Eosin Methylene Blue Agar and Salmonella-Shigella Agar following standard microbiological procedures and then identified/characterized biochemically using commercial API 20E identification kits following the standard manufacturer’s protocol. Isolates were subjected to antibiotic susceptibility testing on Muller Hinton Agar using Kirby Bauer double-disc diffusion technique. The multiple antibiotics resistance index was determined as well. Isolates with reduced susceptibility to Ceftazidime were screened for extended spectrum beta-lactamase, AmpC- and metallo-beta-lactamase-production using Rosco Diagnostic kit.

Results: Sixty-two (100%) Gram-negative bacteria were isolated from a total of 100 samples collected from both sites, out of which 43 (69.4%) are Enterobacteriaceae. A total of 30/43 (69.8%) Enterobacteriaceae including K. pneumoniae, S. enteritica, S. odorifera, E. coli, K. intermediate, P. stuartii, E. aero-
genes, \textit{P. penneri}, \textit{P. mirabilis} and \textit{C. braakii} were recovered from chicken droppings, whereas 13/43 (30.2\%) Enterobacteriaceae including \textit{K. pneumoniae}, \textit{S. enteritica}, \textit{S. odorifera}, \textit{E. coli}, \textit{K. intermediate}, \textit{P. stuartii}, \textit{E. aerogenes}, \textit{P. penneri}, \textit{P. mirabilis} and \textit{C. braakii} were recovered from cow dungs. Two (12.5\%) different isolates demonstrated metallo-beta-lactamase and cephalosporinase (AmpC) production. The isolates were susceptible to six antibiotics tested except Augmentin and Nitrofurantoin where the resistance is 100\% and 85\% respectively while Ceftriaxone and Ofloxacin had the best antibacterial activity against the isolates from both sites. \textbf{Conclusion:} The bacteria of public health importance isolated from these sites and their antibiogram profile have shown the need for proper monitoring and management of animal wastes in order to mitigate the threat to human health in the spirit of One Health as well as contribute to the fight against antibiotic resistance.

\textbf{Keywords}
Antibiogram, Antibiotic Resistance, Enterobacteriaceae, One Health, Nigeria

\section{1. Introduction}
The Enterobacteriaceae, a family of aerobic and Gram-negative rods that naturally inhabit the intestinal tract of humans and animals, have been implicated in many human diseases. This family of bacteria has recently been experiencing a rise in incidence of resistance to antibiotics, as reported in many countries of the world, thus posing a bigger threat to healthcare delivery [1] [2]. They are particularly of clinical importance in the cause of nosocomial and community acquired bacterial infections. With the continued economic activities in poultry practice, cattle ranching and increased exposure of both crop fields and humans to antibiotic-resistant bacteria present in chicken and cow excreta, human contacts with enterobacterial infections are inevitable and have constituted a threat to public health [3].

More so, previous studies have attributed the irrational use of antibiotics in the practice of animal husbandry as the reason for the emergence and spread of resistant bacteria [3] [4]. Resistance of the Enterobacteriaceae to commonly used antibiotics in the last decade has been of an alarming proportion, causing increased public health concerns [5] [6]. The mechanisms of resistance to such antibiotics are usually through efflux pumps, enzyme modification of the antibiotic, selective pressure and antibiotic inactivation [4] [5] [6] [7]. Commonly used antibiotics are becoming less useful owing to resistance and most of the antibiotics considered as last resort are also becoming ineffective for the same reason [8].

Furthermore, carbapenems are \(\beta\)-lactam group of drugs that are currently used as antibiotics of last resort for treating infection because of the problem of multidrug-resistance especially among Gram-negative rods [2] [9] [10]. This re-
sistance has largely been attributed to the production or acquisition of Carbapenemases among enterobacteriaceae family [11]. Originally, organisms belonging to the enterobacteriaceae family were susceptible to carbapenems, but this is no longer true due to the emergence of Carbapenem resistant enterobacteriaceae in the last couple of years and so posing a serious health concern [10]. The Center for Diseases Control and Prevention (CDC) in 2013 reported that Carbapenem-resistant enterobacteriaceae (CRE), which emerged within the past two decades, among other multidrug-resistant organisms, have remained the major cause of untreatable and hard-to-treat infections among hospitalized patients, and are considered an urgent threat to human health [2] [10]. Detecting CRE early in human and animal hosts is highly recommended in controlling infections by them as well as their spread [2].

Consequently, contamination of food and food-producing animals with MDR bacteria harboring MBLs and AmpC enzymes could be a source of antibiotic resistance [12]. Over the last few decades, several extended-spectrum β-lactamases (ESBL) and AmpC-producing Enterobacteriaceae (EPE) have emerged in both human and animal health management globally, with the animals being touted as the transmission link of ESBLs/AmpCs for humans [10] [11] [13]. In addition to this, Ejikeugwu et al., reported AmpC producing Enterobacteriaceae, as well as MDR and production of MBL amongst Klebsiella spp isolated from cow anal swabs in studies carried out in Nigeria [12] [14]. Although some studies have been carried out in Nigeria at poultry and animal houses, yet paucity of data is available on antimicrobial resistance resulting from poultry droppings and cow dungs especially in South-Eastern Nigeria; thus, creating a research gap for this study. In this study, we proposed the hypothesis that antibiotic resistance in Enterobacteriaceae recovered from both poultry droppings and cow dungs could be attributed to intrinsic genetic factors possessed by these organisms that enhance the production of MBL and AmpC enzymes. Hence, this study was aimed at identifying and screening for drug-resistant Enterobacteriaceae isolated from chicken droppings and cow dungs in Onitsha, Anambra state, in the South-Eastern part of Nigeria.

2. Methods

2.1. Study Setting

This is a cross-sectional descriptive study conducted from August, 2020 to April, 2021 at Nkwelle suburb, Onitsha North Local Government Area, Onitsha. Onitsha is a metropolitan city located near the River Niger, in Anambra state, South-Eastern of Nigeria. It lies within 6°10’N 6°47’E coordinates in a 36.12 km² landmass, and has a population of 561,066 [15].

The study included five different poultry and cow ranches with a capacity of 500 birds and 200 cows respectively. Simple random sampling was used to collect swabs of 50 chicken droppings and 50 cows dungs respectively. Information obtained from the farm attendants showed that, although, proper hygiene is
maintained in the poultry and ranch. The livestock are usually given growth enhancement feeds and water containing antibiotics alongside routine veterinary checks, including medical treatments.

**Collection and transportation of fecal Samples**

Four heaped dessert-spoonful of fresh, warm dung pat on the ground samples were collected from adult cattle from 5 different areas where adult cows congregate. They were mixed and then a sterile swab stick was used to rob through the mixture.

Fifty (50) chicken and cow dung swab samples each, were collected from the poultry house and cow ranch in Nkwelle, Onitsha using sterile swab sticks and early in the morning before the attendants came for their routine cleaning and tending of the chicken and cows. Samples were collected by robbing a wet sterile swab stick on chicken and cow excreta dropped at different locations of the same poultry and ranch, for five different places. Each day, the collected samples were transported aseptically and processed within 2 hours of collection. The samples were processed at laboratory in Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Agulu of Nnamdi Azikiwe University, Awka.

**Culture and purification of fecal Samples**

The chicken dropping and cow dung swab samples were each cultured in 5 ml double strength of nutrient broth (CM0003, Oxoid, UK) and incubated overnight at 30˚C. A loopful of the specimen was transferred aseptically onto MacConkey agar (MAC) plates, Eosin Methylene Blue (EMB) agar, Cetrimide selective agar plates, and Salmonella-Shigella agar for the selective isolation of Klebsiella species, Escherichia coli and P. aeruginosa and Salmonella-Shigella species respectively and incubated at 30˚C for 18 - 24 hours for phenotypic characterization. Phenotypically, *Escherichia coli* produces colonies with metallic green sheen on EMB agar and lactose-fermenting colonies on MAC; *Klebsiella* species produce small, circular, elevated and mucoid colony on MAC and non-metallic sheen mucoid colonies on EMB agar while *P. aeruginosa* isolates produce greenish pigmentation on Cetrimide selective agar [16].

**Identification and confirmation of bacterial isolates using BioMerieux API 20E Kit**

After Gram staining and microscopic examination, the various isolates obtained were characterized biochemically using BioMerieux API-20E kit to further identify the organisms to species level. The pure cultures were further characterized using Analytical Profile Index (API) 20E test strip comprising of 20 micro-tubes seeded with dehydrated substrates for enzymes that are produced by Enterobacteriaceae family. To allow for some moisture during incubation, distilled water (5 ml) was spread onto the honey comb wells of the tray to ensure a humid environment in the incubation box before the strips were placed in the tray. Discrete colonies collected from 20 hours overnight culture plates were inoculated into 5 ml API 20E suspension medium, and emulsified to ensure a homogenous bacterial inoculum. Following the manufacturer’s specifications, the
preparations were carefully distributed into the tubes of the strips, ensuring that no air bubbles were left. The tube and cupule for GEL, VP and CIT tests were filled with the suspension while the inoculum was filled up to the tube in the rest of the test segments. To create an anaerobic environment for ADH, LDC, ODC, H2S and URE tests, the tubes were overlaid with mineral oil and the set up incubated for 24 hours at 37˚C. One drop each of TDA and James reagents was added in TDA and IND tubes respectively, one drop each of VP1 and VP2 was added in VP tube after the incubation period. The results were read and recorded after 10 minutes.

**Antimicrobial Susceptibility Testing**

Antimicrobial Susceptibility testing was carried out on all the identified bacterial isolates using the modified Kirby-Bauer disk diffusion method on Mueller-Hinton (MH) agar plates (Oxoid, UK) [2]. The breakpoint chosen was in accordance with the Clinical and Laboratory Standard Institute (CLSI) guideline [13]. The single disks used were: Ofloxacin 5 µg (OFL), Ceftazidime 30 µg (CAZ), Cefuroxime 30 µg (CRX), Gentamicin 10 µg (GEN), Ceftriaxone 30 µg (CTR), Augmentin 30 µg (AUG), Cefixime 5 µg (CXM), Nitrofurantoin 300 µg (NIT).

**Screening for Extended spectrum-β-lactamase production using ROSCO kits**

For the phenotypic detection of the various β-lactamases present in the strains, the double tablet synergy testing using subjective observations of synergy and the combination tablet method was used [13]. Attention was given to the differences in the zones of inhibition. The susceptibility tests were performed following the method M2A6 disc diffusion method on Mueller Hinton agar plate as recommended by the National Committee for Clinical Laboratory Standards [17]. A standardized (0.5 MacFland) inoculum was swabbed onto the Mueller Hinton agar plate using sterile swabs and the discs were aseptically placed on the inoculated plates and pressed firmly onto the agar plate for complete contact while ensuring sufficient space between individual disc to allow for proper measurement of inhibition zones. The test isolates were tested against the following antibiotic discs; Meropenem (MRP10 µg), Meropenem + Cloxacillin (MRPCX), Meropenem + Clavulanate (MRPC), Meropenem + Phenylboronic Acid (MRPBO), Meropenem + Cloxacillin (MRPCX), Meropenem + DPA (MRPDP), Temocillin (30 µg), in an inverted format. The Plates were left on the work table for 30 minutes to allow for pre-diffusion of antibiotics into the agar. Afterwards, they were incubated at 37˚C for 18 - 24 hours. The susceptibility of each isolate to each antibiotic was shown by a clear zone of growth inhibition and this was measured using a meter rule in millimeters and the diameter of the zones of inhibition was then interpreted following the guide stated by the manufacturer.

**Screening for Amp-C β-lactamase production**

To test for Amp-C β-lactamase production, the test organisms were screened for presumptive AmpC production by testing their susceptibility to cefoxitin (30 µg...
μg) using Kirby Bauer disk diffusion. Following CLSI standard, isolates with an IZD of ≤ 18 mm were suspected to produce AmpC enzyme.

**Screening for Metallo-β-lactamase (MBL) production**

This was done by phenotypically screened for the production of MBL in the test isolates. Their susceptibility to imipenem (IPM), meropenem (MEM), and ertapenem (ETP) was also done according to the CLSI criteria. Test isolates with an inhibition zone diameter (IZD) of ≤23 mm were suspected to harbor MBL enzyme.

**Determination of Multiple Antibiotic Resistance Index (MARI)**

The MARI was calculated using the formula:

\[
\text{MARI} = \frac{\text{Number of antibiotics to which the isolates were resistant}}{\text{Total number of antibiotics to which the isolates were subjected}}
\]

The incidence of multidrug resistant isolates was calculated from the formula [15]

\[
\text{Incidence of multidrug resistant isolates} = \frac{\text{Number of isolates with MARI} \geq 0.3}{\text{Total number of Isolates}} \times 100
\]

**2.2. Data Analysis**

All the data collected was summarized and tabulated using Microsoft excel software 2016. The results were calculated using percentages and presented in tables, while the Multiple Antibiotic Resistance index (MAR index) was calculated for each isolate and tabulated in the result section.

**3. Results**

A total of 43 (69.4%) Enterobacteriaceae recovered from this study out of 62 (100%) Gram-negative bacteria (GNB) isolates obtained from this study. **Table 1** shows the frequency of Gram-negative bacteria recovered from the samples collected from 40 (80%) chicken droppings and 22 (44%) cow dungs. *K. pneumoniae* and *E. coli* had the highest frequency in chicken droppings (15%), while *K. pneumoniae* and *E. cloacae* had the highest frequency in cow dung (18.1%). The Enterobacteriaceae recovered all showed to be lactose fermenters with pink colonies on MAC, whereas non-lactose fermenters showed pale colonies on MAC and were all negative for oxidase test. Characterization of the isolates biochemically using the API®20E kit identified the isolates accordingly (**Table 1**).

**Table 2** and **Table 3** show the antibiogram profiles of the isolates to the tested antibiotics together with their respective MARI. All the isolates from chicken dropping, except *Citrobacter braaki*, were resistant to Augmentin while the isolates *Serratia odorifera* and *Enterobacter cloacae* were resistant to all the antibiotics tested (**Table 2**). Ceftriaxone and Ofloxacin had the best antibacterial activity against the isolates from both sites. All the isolates from cow dungs were resistant to Augmentin while *Shewanella putrefaciens* was resistant to all the antibiotics tested (**Table 2**). **Table 2** and **Table 3** show the multidrug resistant
Table 1. Frequency of isolates identified from this study using API-20E kit.

| Isolates’ Source | Assigned Isolate API Number | Organisms                          | *Frequency (%) |
|------------------|-----------------------------|------------------------------------|----------------|
| Chicken Droppings (N = 40) | 7214773 | *Klebsiella Pneumoniae* | 15.0 |
|                   | 5506572 | *Salmonella enterica spp arizona* | 2.5 |
|                   | 402000  | *Shewanella putrefaciens* | 7.5 |
|                   | 3304573 | *Enterobacter cloacae* | 7.5 |
|                   | 1504573 | *Citrobacter braaki* | 2.5 |
|                   | 5105532 | *Escherichia coli* | 15.0 |
|                   | 536000  | *Proteus mirabilis* | 5.0 |
|                   | 5737773 | *Serratia odorifera* | 10.0 |
|                   | 206020  | *Burkholderia cepacia* | 7.5 |
|                   | 1104572 | *Kluyvera intermedia* | 7.5 |
|                   | 224300  | *Providencia stuartii* | 2.5 |
|                   | 604040  | *Acinetobacter baumannii* | 2.5 |
|                   | 1205773 | *Pantoea spp* | 7.5 |
|                   | 1305773 | *Enterobacter aerogenes* | 5.0 |
|                   | 436020  | *Proteus penneri* | 2.5 |

| Isolates’ Source | Assigned Isolate API Number | Organisms                          | *Frequency (%) |
|------------------|-----------------------------|------------------------------------|----------------|
| Cow Dung (N = 22) | 5214773 | *Klebsiella pneumoniae* | 18.2 |
|                   | 2302000 | *Pseudomonas aeruginosa* | 4.5 |
|                   | 0624300 | *Providencia stuartii* | 4.5 |
|                   | 3305573 | *Enterobacter cloacae* | 18.2 |
|                   | 1205000 | *Ewingella Americana* | 9.1 |
|                   | 1144572 | *Escherichia coli* | 4.5 |
|                   | 5304773 | *Serratia fonticola* | 9.1 |
|                   | 1205773 | *Pantoea spp* | 13.6 |
|                   | 1206773 | *Serratia ficaria* | 4.5 |
|                   | 0402000 | *Shewanella putrefaciens* | 9.1 |
|                   | 0306000 | *Burkholderia cepacia* | 4.5 |

*Frequency = adjusted to 1 decimal place.

Table 2. Antibiogram of the isolates from the chicken droppings.

| Isolates                | Antibiotics IZD (mm) | *MARI |
|-------------------------|----------------------|-------|
|                         | CAZ     | CRX    | GEN    | CXM    | OFL    | AUG    | NIT    | CTR    |
| *Escherichia coli*      | 0       | 0      | 16     | 0      | 12     | 0      | 0      | 25     | 0.750  |
| *K. pneumoniae*         | 12      | 0      | 18     | 12     | 20     | 0      | 20     | 30     | 0.375  |
| *Burkholderia cepacia*  | 0       | 0      | 13     | 0      | 21     | 0      | 0      | 25     | 0.625  |
| *Kluyvera intermedia*   | 20      | 15     | 20     | 0      | 28     | 0      | 22     | 30     | 0.375  |
| Bacterial Species                  | OFL | CAZ | CRX | GEN | CTR | AUG | CXM | NIT | MARI |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|------|
| Enterobacter cloacae              | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 1.000|
| Shewanella putrefaciens           | 0   | 0   | 11  | 0   | 20  | 0   | 0   | 0   | 0.750|
| Enterobacter aerogenes            | 20  | 15  | 0   | 25  | 20  | 0   | 15  | 18  | 0.500|
| Citrobacter braakii               | 0   | 10  | 20  | 0   | 18  | 19  | 21  | 25  | 0.375|
| Proteus mirabilis                 | 18  | 12  | 15  | 28  | 25  | 0   | 0   | 25  | 0.500|
| Serratia odorifera                | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 1.000|
| Pantoea spp                       | 0   | 0   | 0   | 0   | 20  | 0   | 11  | 20  | 0.750|
| Acinetobacter baumannii           | 0   | 0   | 18  | 0   | 30  | 0   | 0   | 0   | 0.625|
| Proteus penneri                   | 0   | 0   | 19  | 0   | 25  | 0   | 18  | 20  | 0.500|
| Providencia stuartii              | 20  | 19  | 12  | 15  | 28  | 0   | 10  | 30  | 0.500|
| Salmonella enteric spp Arizona    | 0   | 0   | 0   | 0   | 0   | 0   | 20  | 0   | 0.875|
| E. coli                          | 20  | 13  | 0   | 22  | 15  | 0   | 15  | 20  | 0.625|
| K. pneumoniae                     | 20  | 9   | 11  | 20  | 18  | 0   | 0   | 15  | 0.500|
| Burkholderia cepacian             | 16  | 15  | 19  | 24  | 21  | 0   | 25  | 22  | 0.250|
| Kluyvera intermedia               | 18  | 10  | 18  | 20  | 20  | 0   | 25  | 21  | 0.250|
| Enterobacter cloacae              | 17  | 0   | 14  | 13  | 20  | 0   | 0   | 20  | 0.625|
| Shewanella putrefaciens           | 25  | 15  | 0   | 20  | 20  | 0   | 12  | 20  | 0.375|
| Enterobacter aerogenes            | 20  | 15  | 18  | 22  | 20  | 0   | 19  | 21  | 0.250|
| Proteus mirabilis                 | 26  | 0   | 20  | 30  | 30  | 0   | 20  | 30  | 0.250|
| Serratia odorifera                | 18  | 15  | 0   | 30  | 0   | 0   | 10  | 0   | 0.625|
| Pantoea spp                       | 0   | 0   | 18  | 0   | 28  | 0   | 0   | 30  | 0.625|
| E. coli                          | 20  | 17  | 0   | 30  | 20  | 0   | 22  | 25  | 0.250|
| K. pneumoniae                     | 15  | 14  | 0   | 22  | 0   | 0   | 11  | 0   | 0.750|
| Burkholderia cepacian             | 0   | 0   | 18  | 20  | 30  | 0   | 25  | 30  | 0.375|
| Kluyvera intermedia               | 25  | 20  | 0   | 32  | 0   | 0   | 17  | 0   | 0.500|
| Enterobacter cloacae              | 13  | 0   | 12  | 22  | 0   | 0   | 12  | 0   | 0.875|
| Shewanella putrefaciens           | 15  | 0   | 20  | 12  | 10  | 0   | 15  | 20  | 0.750|
| Serratia odorifera                | 21  | 18  | 10  | 25  | 20  | 0   | 20  | 15  | 0.250|
| Pantoea spp                       | 14  | 0   | 18  | 24  | 12  | 0   | 15  | 22  | 0.625|
| E. coli                          | 22  | 12  | 0   | 27  | 18  | 0   | 10  | 20  | 0.500|
| K. pneumoniae                     | 18  | 0   | 20  | 20  | 23  | 0   | 22  | 28  | 0.250|
| Serratia odorifera                | 20  | 0   | 15  | 0   | 20  | 0   | 0   | 27  | 0.625|

KEY: OFL = Ofloxacin 5 µg; CAZ = Ceftazidime 30 µg; CRX = Cefuroxime = 30 µg; GEN = Gentamicin 10 µg; CTR = Ceftriaxone 30 µg; AUG = Augmentin 30 µg; CXM = Cefixime 5 µg; NIT = Nitrofurantoin 300 µg; *MARI = adjusted to 3 decimal places.
Table 3. Antibiogram of the isolates from the cow dungs.

| Bacterial Isolates       | Antibiotics IZD (mm)                  | *MARI     |
|--------------------------|--------------------------------------|-----------|
|                           | CAZ CRX GEN CXM OFL AUG NIT CTR      |           |
| *E. coli*                | 15 10 20 15 28 0 18 35 0.500          |           |
| *K. pneumoniae*          | 0 0 15 0 22 0 0 22 0.750              |           |
| *Enterobacter cloacae*   | 20 20 20 25 30 0 20 30 0.875          |           |
| *Pantoea spp*            | 0 0 18 0 30 0 0 30 0.625              |           |
| *Ewingella Americana*    | 20 19 20 25 22 0 23 25 0.125          |           |
| *Serratia fonticola*     | 20 17 0 28 21 0 20 27 0.250           |           |
| *Shewanella putrefaciens*| 0 0 0 0 0 0 0 1.000                   |           |
| *Serratia ficaria*       | 15 0 20 20 25 0 25 28 0.375           |           |
| *P. aeruginosa*          | 0 0 12 0 20 0 0 30 0.750              |           |
| *Providencia stuartii*   | 20 22 20 30 28 0 11 30 0.250          |           |
| *Burkholderia cepacia*   | 0 0 0 0 21 0 0 25 0.750              |           |
| *K. pneumoniae*          | 20 13 15 25 0 0 0 15 0.750            |           |
| *Enterobacter cloacae*   | 19 13 20 15 20 0 17 20 0.375          |           |
| *Pantoea spp*            | 0 0 0 0 25 13 0 20 0.750              |           |
| *Ewingella Americana*    | 16 14 17 20 18 0 14 20 0.375          |           |
| *Serratia fonticola*     | 20 20 0 25 21 0 17 23 0.250           |           |
| *Shewanella putrefaciens*| 20 0 20 25 10 0 20 0 0.500            |           |
| *K. pneumoniae*          | 20 0 0 30 22 0 18 20 0.375            |           |
| *Enterobacter cloacae*   | 15 0 20 12 10 0 15 20 0.750           |           |
| *Pantoea spp*            | 20 13 0 30 17 0 20 25 0.375           |           |
| *K. pneumoniae*          | 18 0 20 15 20 0 0 0 0.625              |           |
| *Enterobacter cloacae*   | 17 15 20 17 20 0 15 20 0.375           |           |

KEY: OFL = Ofloxacin 5 µg; CAZ = Ceftazidime 30 µg; CRX = Cefuroxime = 30 µg; GEN = Gentamicin 10 µg; CTR = Ceftriaxone 30 µg; AUG = Augmentin 30 µg; CXM = Cefixime 5 µg; NIT = Nitrofurantoin 300 µg; *MARI = adjusted to 3 decimal places.

profile of the isolates with most of the isolates showing resistance to three or more antibiotics (above 0.2) hence are considered as multi-antibiotics resistant strains. Table 4 shows the results of the screening tests for AmpC production among the 16 MDR isolates. From the test, Escherichia coli, Proteus penneri, Pantoea spp, Shewanella putrefaciens were positive for AmpC production. The results of the screening tests for Metallo-β-lactamase (MBL) production (Table 5) among the 16 multidrug-resistant isolates revealed that Serratia odorifera and Enterobacter cloacae were positive for Metallo-β-lactamase (MBL) production. Table 6 shows the results of the screening tests for possible ESBL production.
Table 4. AmpC production by the multi drug resistant isolates.

| Enterobacteriaceae Isolates                  | CTX CX (mm) | CTX 30 (mm) | RESULT |
|---------------------------------------------|-------------|-------------|--------|
| *Acinetobacter baumannii*                  | 18          | 18          | -      |
| *Pantoea spp*                               | 30          | 30          | -      |
| *Salmonella enterica spp. Arizona*          | 30          | 30          | -      |
| *Pantoea spp*                               | 22          | 20          | -      |
| *Burkholderia cepacia*                      | 25          | 22          | -      |
| *Serratia odorifera*                        | 22          | 16          | -      |
| *Escherichia coli*                          | 30          | 25          | +      |
| *Shewanella putrefaciens*                   | 25          | 23          | -      |
| *Enterobacter cloacae*                      | 17          | 18          | -      |
| *Proteus penneri*                           | 27          | 0           | +      |
| *Burkholderia cepacia*                      | 25          | 25          | -      |
| *Pantoea spp*                               | 22          | 18          | -      |
| *Pseudomonas aeruginosa*                    | 27          | 28          | -      |
| *Pantoea spp*                               | 22          | 12          | +      |
| *Shewanella putrefaciens*                   | 20          | 15          | +      |
| *Pantoea spp*                               | 25          | 28          | -      |

Table 5. Metallo–B-lactamase (MBL) production by the multi drug resistant isolates.

| SAMPLE CODE                        | MRPDP (mm) | MRP10 (mm) | RESULT |
|------------------------------------|------------|------------|--------|
| *Acinetobacter baumannii*          | 25         | 25         | -      |
| *Pantoea spp*                      | 28         | 28         | -      |
| *Salmonella enterica spp. Arizona* | 30         | 30         | -      |
| *Pantoea spp*                      | 28         | 30         | -      |
| *Burkholderia cepacia*             | 30         | 30         | -      |
| *Serratia odorifera*               | 22         | 0          | +      |
| *Escherichia coli*                 | 26         | 30         | -      |
| *Shewanella putrefaciens*          | 35         | 32         | -      |
| *Enterobacter cloacae*             | 18         | 10         | +      |
| *Proteus penneri*                  | 30         | 32         | -      |
| *Burkholderia cepacia*             | 30         | 30         | -      |
| *Pantoea spp*                      | 28         | 30         | -      |
| *Pseudomonas aeruginosa*           | 30         | 30         | -      |
| *Pantoea spp*                      | 22         | 18         | -      |
| *Shewanella putrefaciens*          | 18         | 15         | -      |
| *Pantoea spp*                      | 30         | 28         | -      |
Table 6. ESBL production by the multi-drug resistant isolates.

| Enterobacteriaceae Isolates                  | Positive for ESBL | Negative for ESBL | Total |
|---------------------------------------------|-------------------|-------------------|-------|
| Acinetobacter baumannii                    | 0                 | 1                 | 1     |
| Pantoea spp                                | 0                 | 5                 | 5     |
| Salmonella enterica app Arizona             | 0                 | 1                 | 1     |
| Burkholderia cepacia                       | 0                 | 1                 | 1     |
| Serratia odorifera                         | 0                 | 2                 | 2     |
| Escherichia coli                           | 0                 | 1                 | 1     |
| Shewanella putrefaciens                    | 0                 | 2                 | 2     |
| Enterobacter cloacae                        | 0                 | 1                 | 1     |
| Proteus penneri                            | 0                 | 1                 | 1     |
| Pseudomonas aeruginosa                     | 0                 | 1                 | 1     |
| Total                                      | 0                 | 16                | 16    |

among the 16 multidrug-resistant isolates. The result showed that none of the isolates were positive for ESBL production.

4. Discussion

The nonstop incidence of antimicrobial resistance caused by selective pressure, continued abuse and misuse of antibiotics and the use of antibiotics in animal husbandry have created a very serious problem in the treatment of bacterial infections and has become a daunting task for public health practitioners worldwide since only a very few antibiotics are effective for use. The increasing emergence of multidrug resistance is making the issue more problematic.

In this study, we investigated the presence, identity and antimicrobial profile of Enterobacteriaceae isolated from chicken and cow dung in Onitsha, Anambra State, Nigeria. The results revealed the presence of forty three (69.4%) enterobacteriaceae. This demonstrates their dominance among the 62 gram negative isolates recovered. E. coli (15%) and Klebsiella pneumoniae (15%) were bacteriologically recovered from the chicken droppings swab samples as the most prevalent organisms which is in tandem with the report of Ejikeugwu et al. [14] who isolated similar organisms from anal swap samples from abattoir. However, Klebsiella pneumoniae and Enterobacter cloacae were the most prevalent Enterobacteriaceae isolated from cow dung with a percentage of 18.2% each. Both bacteriological recoveries (chicken droppings and cow dung) agree with the findings of Amador et al. [3] in Portugal who isolated various Enterobacteriaceae from Portuguese livestock manure. These bacteria are members of the Enterobacteriaceae family and part of the human normal flora.

The evaluation of the antimicrobial resistance profiles of the recovered isolates under study is paramount as antibiotic-resistant bacteria in animal excreta are
an emergent concern. The resistance profiles of all Enterobacteriaceae isolates were evaluated by exposure to eight antibiotics of four different classes for the phenotypic characterization of the isolates. These were chosen to represent the main antibacterial classes used in human medicine and livestock production in Nigeria. The resistance of all isolates from chicken dropping, except *Citrobacter braaki*, to Augmentin as well as the resistance of *Serratia odorifera* and *Enterobacter cloacae* to all the antibiotics tested can be attributed to the use of extended spectrum cephalosporins such as cefotaxim and cefotazim in livestock. The results of this research buttress the findings of [11] who reported that the presence of Carbapenemases producing Enterobacteriaceae in animals is becoming worrisome. These organisms, all of which are of public health importance, are in line with the report [2] who reported that Carbapenem-resistant Enterobacteriaceae are organisms of medical importance. As well as [18] in Southern China who recorded high level of Carbapenem-resistant *Acinetobacter* spp. from clinical infection and fecal survey samples in Portugal [3]. The antibiogram profiles of the bacterial isolates from both sites revealed that some of the bacterial isolates were highly resistant to commonly used agents although some of the isolates were sensitive to Ofloxacin and moderately sensitive to Ceftazidime. Most of the isolates were resistant to three or more antibiotic classes hence are considered as multi-antibiotics resistant strains. The results of isolates from chicken dropping show that only one isolate, *Providencia stuartii*, gave a MARI of <0.20 as it had a MARI of 0.13 with others having 0.20 and above while *Ewingella americana* and *Providencia stuartii* both had a MARI of 0.13 among organisms isolated from the cow dung. The multiple antibiotics resistance index (MARI) is a protocol used to explain the spread of bacteria resistance and resistant genes in any bacterial population [19] [20]. Generally, Multiple antibiotics resistance index above 0.20 means that bacteria isolates originating from such an environment has been exposed to indiscriminate use of several antibiotics in time past [19] [21]. The multi-drug resistance to the antibiotics of different classes observed in this study may be due to the increasing administration of quinolones to treat avian infections [3]. The unnecessary use of antibiotics for enhancement of growth and prevention of diseases in farm animals has impressed selective pressures that induce more resistance among bacteria in the community. From the sixteen isolates examined phenotypically for the production of metallo β-lactamase (MBL), two (12.5%) isolates were positive for the production of this enzyme. The production of MBL in this study is similar to a previous study conducted by Ejikeugwu et al. [12] in which MBL was detected in *Klebsiella* species isolated from cow anal swabs. Only two AmpC producing Enterobacteriaceae was detected when the isolates were phenotypically screened for the enzyme. This is similar to an earlier report by Ejikeugwu et al. [14] in which AmpC enzymes were significantly detected in the *E. coli* and *Klebsiella* species isolated from cow anal swabs from an abattoir in Abakaliki, Nigeria. These results illustrate that the AmpC and metallo β-lactamase (MBL) producing species isolated in this study are multi-drug resistant. They also produce AmpC and metallo β-lactamase (MBL) en-
zymes which allow them to be resistant to the 2nd and 3rd generation cephalosporins which are clinically used to manage and treat serious bacterial infections. Furthermore, all the isolates screened phenotypically for ESBL production showed negative, this is in contrast to the review done by Madec et al. [11] where a study conducted among 699 S. enterica isolates from 1152 retail chickens reported a 24.6% rate of ESBL producers in Shanghai, China. This current study is relevant and a springboard to the increasing prevalence of antibiotic resistance in the non-nosocomial environment such as abattoir. More so, it provides acceptance to the possible abuse and irrational use of antibiotics in animal husbandry and for other non-clinical purposes. Hitherto, fecal excrement of chicken and cows in constant contact with humans who are husbandry rearers, pose high risk of cross contamination and further affects the antimicrobial resistance status and ultimately, the public health standards of nations.

Study Limitations: The molecular characterization of the isolates to further confirm the identity of the isolates wasn’t conducted at the time of this writing due to limited funding. Also, the genes responsible for drug resistance could not be identified for the same financial constraints.

5. Conclusions

The bacteria of public health importance isolated from these sites and their antibiogram profile have shown the need for proper monitoring and management of animal wastes in order to mitigate the threat to human health in the spirit of One Health as well as contribute to the fight against antibiotic resistance.

What is known about this topic?

World over:

- Resistance of the Enterobacteriaceae to commonly used antibiotics in the last decade has been of an alarming proportion, causing increased public health concerns.
- The last few years have witnessed the proliferation of several extended-spectrum β-lactamases (ESBL) and AmpC-producing Enterobacteriaceae (EPE) in both human and animal health management globally.
- Animals have been touted as the transmission link of ESBLs/AmpCs for humans and demanded urgent response.

What this study adds

This study has showed that:

- There is the presence of resistant strains among enterobacteriaceae found in chicken and cow droppings.
- The need for proper monitoring and management of animal wastes in order to mitigate the threat to human health in the spirit of One Health.
- More evidence that animals could serve as the transmission link of ESBLs/AmpCs in humans.

Acknowledgements

This research was conducted by a project group between the Department of
Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Anambra state Nigeria and the Physician Assistantship/Public Health Department of Central University, Accra, Ghana. We would like to acknowledge all members of our team and staff of the laboratory for their continuous commitment to our research efforts.

**Conflicts of Interest**

None to declare.

**Funding**

The study did not receive external funding; instead, it was self-funded.

**Authors’ Contributions**

CCA initiated the concept of the research, performed the Lab work and wrote the first draft. ANO elaborated the idea, designed the work and as well as supervised the lab work. PAE and MUO helped with laboratory works while JK participated in writing the manuscript. All the authors have read and agreed to the final manuscript.

**References**

[1] Almugadam, B.S., Osman, N.A., Alaaeldeen, B.A., Ahmed, B.E. and Wang, L. (2018) Prevalence and Antibiotics Susceptibility Patterns of Carbapenem Resistant *Enterobacteriaceae*. *Journal of Bacteriology and Mycology*, 6, 187-190. [https://doi.org/10.15406/jbmoa.2018.06.00201](https://doi.org/10.15406/jbmoa.2018.06.00201)

[2] Oli, A.N., Itumo, C.J., Okam, P.C., Ezebialu, I.U., Okeke, K.N., Ifezulike, C.C., Ezeobi, I., Emechebe, G.O., Okezie, U.M., Adejumo, S.A. and Okoyeh, J.N. (2019) Carbapenem-Resistant *Enterobacteriaceae* Posing a Dilemma in Effective Healthcare Delivery. *Antibiotics*, 8, Article No. 156. [https://doi.org/10.3390/antibiotics8040156](https://doi.org/10.3390/antibiotics8040156)

[3] Amador, P., Fernandes, R., Prudêncio, C. and Duarte, I. (2019) Prevalence of Antibiotic Resistance Genes in Multidrug-Resistant *Enterobacteriaceae* on Portuguese Livestock Manure. *Antibiotics*, 8, Article No. 23. [https://doi.org/10.3390/antibiotics8010023](https://doi.org/10.3390/antibiotics8010023)

[4] Van den, H.M.S., Gouws, P.A. and Hoffman, I.C. (2018) Importance and Implications of Antibiotic Resistance Development in Livestock and Wildlife Farming in South Africa: A Review. *South African Journal of Animal Science*, 48, 401-412. [https://doi.org/10.4314/sajas.v48i3.1](https://doi.org/10.4314/sajas.v48i3.1)

[5] Moravej, H., Zahra, M., Maryam, Y., Mohammad, H., Ali, M., Mehrcdn, M.M. and Reza, M. (2018) Antimicrobial Peptides: Features, Action, and Their Resistance Mechanisms in Bacteria. *Microbial Drug Resistance*, 24, 747-767. [https://doi.org/10.1089/mdr.2017.0392](https://doi.org/10.1089/mdr.2017.0392)

[6] Chandra, H., Bishnoi, P., Yadav, A., Patni, B., Mishra, A. and Nautiyal, A. (2017) Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials—A Review. *Plants*, 6, Article No. 16. [https://doi.org/10.3390/plants6020016](https://doi.org/10.3390/plants6020016)

[7] Oli, A.N., Eze, D.E., Gugu, T.H., Ezeobi, I., Maduagwu, U.N. and Ihekwereme, C.P. Multi-Antibiotic Resistant Extended-Spectrum Beta-Lactamase Producing Bacteria Pose a Challenge to the Effective Treatment of Wound and Skin Infections. *The Pan*
African Medical Journal, 27, Article No. 66.

[8] Zhang, R., Liu, L., Zhou, H., Waichi, E., Li, J., Fang, Y., Li, Y., Liao, K. and Chen, S. Nationwide Surveillance of Clinical Carbapenem-Resistant Enterobacteriaceae (CRE) Strains in China. EBioMedicine, 19, 98-106. https://doi.org/10.1016/j.ebiom.2017.04.032

[9] Ali, M., Amal, J., Mohammed, A., Wael, M., Merin, G., Sadananda, A., Sanjay, R., Darshan, D.D., Chitra, J., Saijith, V., Aftab, A.K., Jilani, S. and Poojdev, J. (2020) Antimicrobial Resistance, Mechanisms and Its Clinical Significance. Disease A-Month, 66, Article ID: 100971

[10] Hosurur Subramanya, S., Bairy, I., Nayak, N., Amberpet, R., Padukone, S., Metok, Y., Bhatta, D.R. and Sahith, B. (2020) Detection and Characterization of ESBL-Producing Enterobacteriaceae from the Gut of Healthy Chickens, Gallus gallus domesticus in Rural Nepal: Dominance of CTX-M-15-Non-ST131 Escherichia coli Clones. PLoS ONE, 15, e0227725. https://doi.org/10.1371/journal.pone.0227725

[11] Madec, J., Haenni, M., Nordmann, P. and Poiriel, L. (2017) Extended-Spectrum β-Lactamase/AmpC- and Carbapenemase-Producing Enterobacteriaceae in Animals: A Threat for Humans? Clinical Microbiology and Infection, 23, 826-833. https://doi.org/10.1016/j.cmi.2017.01.013

[12] Ejikeugwu, C., Duru, C., Eluu, S., Oguejiofor, B., Ezeador, C., Ogene, L. and Iroha, I. (2017) Isolation and Phenotypic Detection of Metallo-Beta-Lactamase (MBL)-Producing Klebsiella Species from Cow Anal Swabs. Global Journal of Pharmacy & Pharmaceutical Sciences, 2, 54-58. https://doi.org/10.19080/GJPPS.2017.02.555586

[13] Hansen, F., Hammerum, A.M., Skov, R.L., Giske, C.G., Sundsfjord, A. and Samuelsen, O. (2012) Evaluation of Rosco Neo-Sensitabs for Phenotypic Detection and Subgrouping of ESBL-, AmpC- and Carbapenemase-Producing Enterobacteriaceae. APMIS, 120, 724-732. https://doi.org/10.1111/j.1600-0463.2012.02898.x

[14] Ejikeugwu, C., Nworie, O., Agah, M.V., Oguejiofor, B., Ovia, K., Nworie, C.O., Iwunze, A.C., Nwambike, A. and Edeh, C. (2018) Bacteriological and Antibiogram of AmpC Producing Enterobacteriaceae Isolated from Abattoir. Microbiology: Current Research, 2, 37-41. http://www.alliedacademies.org/microbiology-current-research/

[15] Federal Republic of Nigeria (2010) 2006 Population and Housing Census: Priority Table, Volume III. National Population Commission: Population Distribution by Sex, State, LGA & Senatorial District, Abuja.

[16] Laine, L., Perry, J.D., Lee, J., Oliver, M., James, A.L., De La Foata, C., Halimi, D., Orenge, S., Galloway, A. and Gould, F.K. (2009) Isolation of Pseudomonas aeruginosa from the Sputa of Cystic Fibrosis Patients. Journal of Cystic Fibrosis, 8, 143-149. https://doi.org/10.1016/j.jcf.2008.11.003

[17] Clinical and Laboratory Standards Institute (CLSI) (2017) Performance Standards for Antimicrobial Disk Susceptibility Tests. 12th Edition, Clinical and Laboratory Standards Institute, Wayne, PA.

[18] Li, S., Duan, X., Peng, Y. and Rui, Y. (2019) Molecular Characteristics of Carbapenem-Resistant Acinetobacter spp. from Clinical Infection Samples and Fecal Survey Samples in Southern China. BMC Infectious Diseases, 19, Article No. 900. https://doi.org/10.1186/s12879-019-4423-3

[19] Ejiofor, O.S., Ajunwa, O.M., Ezeudu, C.E., Emechebe, G.O., Okeke, K.N., Ifezulike, C.C., Ekejindu, I.M., Okoye, J.N., Osuala, E.O. and Oli, A.N. (2018) The Bacteriology and Its Virulence Factors in Neonatal Infections: Threats to Child Survival Strategies. Journal of Pathogens, 2018, Article ID: 4801247.
[20] Hosu, M.C., Vasaikar, S., Okuthe, G.E. and Apalata, T. (2021) Molecular Detection of Antibiotic-Resistant Genes in Pseudomonas aeruginosa from Nonclinical Environment: Public Health Implications in Mthatha, Eastern Cape Province. *South Africa. International Journal of Microbiology*, 2021, Article ID: 8861074. https://doi.org/10.21203/rs.3.rs-31330/v1

[21] Ejiofor, S.O., Edeh, A.D., Ezeudu, C.E., Gugu, T.H. and Oli, A.N. (2016) Multi-Drug Resistant Acute Otitis Media amongst Children Attending Out-Patient Clinic in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, South-East Nigeria. *Advances in Microbiology*, 6, 495-501. http://www.scirp.org/journal/aim
https://doi.org/10.4236/aim.2016.67049
## Appendix

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

| Item No | Recommendation | Page No |
|---------|----------------|---------|
| **Title and abstract** | 1  | 1) Indicate the study’s design with a commonly used term in the title or the abstract  
2) Provide in the abstract an informative and balanced summary of what was done and what was found | 1 |
| **Introduction** | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 2 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 2 |
| **Methods** | | |
| Study design | 4 | Present key elements of study design early in the paper | 3 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 3 |
| Participants | 6 | 1) Give the eligibility criteria, and the sources and methods of selection of participants | NA |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | NA |
| Data sources/measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | NA |
| Bias | 9 | Describe any efforts to address potential sources of bias | NA |
| Study size | 10 | Explain how the study size was arrived at | NA |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | NA |
| | | 1) Describe all statistical methods, including those used to control for confounding | NA |
| | | 2) Describe any methods used to examine subgroups and interactions | NA |
| Statistical methods | 12 | 3) Explain how missing data were addressed | NA |
| | | 4) If applicable, describe analytical methods taking account of sampling strategy | NA |
| | | 5) Describe any sensitivity analyses | NA |
| **Results** | | |
| Participants | 13* | 1) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed | NA |
| | | 2) Give reasons for non-participation at each stage | NA |
| | | 3) Consider use of a flow diagram | NA |
Continued

| Section               | Item | Description                                                                                                                                  | Notes |
|-----------------------|------|----------------------------------------------------------------------------------------------------------------------------------------------|-------|
| Descriptive data      | 14*  | 1) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders     | NA    |
|                       |      | 2) Indicate number of participants with missing data for each variable of interest                                                          |       |
| Outcome data          | 15*  | Report numbers of outcome events or summary measures                                                                                       | 6     |
|                       |      | 1) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 12    |
| Main results          | 16   | 2) Report category boundaries when continuous variables were categorized                                                                      | 12    |
|                       |      | 3) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period                                   | 12    |
| Other analyses        | 17   | Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses                                               | 12    |

**Discussion**

| Section               | Item | Description                                                                                                                                  | Notes |
|-----------------------|------|----------------------------------------------------------------------------------------------------------------------------------------------|-------|
| Key results           | 18   | Summarise key results with reference to study objectives                                                                                   | 7     |
| Limitations           | 19   | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 7     |
| Interpretation        | 20   | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 7     |
| Generalizability      | 21   | Discuss the generalizability (external validity) of the study results                                                                       | NA    |

**Other information**

| Section               | Item | Description                                                                                                                                  | Notes |
|-----------------------|------|----------------------------------------------------------------------------------------------------------------------------------------------|-------|
| Funding               | 22   | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 9     |

NA: Not applicable. *Give information separately for exposed and unexposed groups. Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at [http://www.plosmedicine.org/](http://www.plosmedicine.org/), Annals of Internal Medicine at [http://www.annals.org/](http://www.annals.org/), and Epidemiology at [http://www.epidem.com/](http://www.epidem.com/)). Information on the STROBE Initiative is available at [http://www.strobe-statement.org/](http://www.strobe-statement.org/).

**List of Abbreviations**

- MARI: Multiple Antibiotics Resistance Index
- MBL: Metallo-β-lactamase
- API: Analytical Profile Index
- CRE: Carbapenem-resistant Enterobacteriaceae
- MAC: MacConkey Agar
- SSA: Salmonella-Shigella Agar
- EMB: Eosin Methylene Blue Agar
- GNB: Gram Negative Bacteria