Development of a robust model for detecting and classifying multidrug resistance and its evaluation through antibiogram studies on selected gram negative bacteria from Katsina Metropolis, Katsina State, Nigeria

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Abstract: Antimicrobial Resistance (AMR) threatens humanity. Curtailing AMR necessitates periodic re-evaluation of resistance patterns andupdated reporting of resistant pathogens in various locales. Here, we adapted definitions from an international experts' guidance report by CDC/ECDC(withCLSI/EUCAST& FDA's contributions) and developed a robust modeladapted to the Nigerian context, for classifying antibiotic-resistant bacteria into multi/extensively drug-resistant subtypes. We inferred from the report the redundancy ofexclusive use of Multiple Antibiotic Resistance Index/MARI asan indicator of multi/extensive drug resistance; hence, we devised additional indexes: Multiple Antibiotic Non-Sustainability Index/MANSI and Class Non-Susceptibility Index/CNSIas viable alternatives. Weevaluated themodel by ascertaining multidrug resistancefromsomecommonly isolated Gram-negative bacteria originating from medical/food/environmental samples from Katsina Metropolis, Nigeria;maintained in the Microbiology Lab, Umaru Musa 'Yar'adua University, Katsina, Nigeria (2018-2020); using the Kirby-Bauer technique.The model showed that four out of five bacteria (80%) are multidrug resistant: *Enterobacteraerogenes, Klebsiellapneumoniae, Pseudomonas aeruginosa* and*Shigelladysentriae*.Statistical analyses showed that inhibition zones elicited by the various antibioticsdiffer significantly from each other at $p \leq 0.05$ ($p = 0.0025$; $F_{cal} = 4.30$, $F_{crit} = 2.36$), and also amongst the tested bacteria ($p = 0.000081$, $F_{cal} = 11.32$, $F_{crit} = 3.01$).TheCNSIs(ranging from 0/8 to 5/8) proved the best technique for detecting multi/extensive drug-resistance than the MARIs(0.0-0.5) and the MANSIs(0.0-0.6). The model also shows the percentage effectiveness of the antibiotics at a glance, with cephalexin/ceporex (80% resistance), Pefloxacin (60% resistance), and Augmentin, Ciprofloxacin, and Nalidixic Acid (40% resistance each) being highly resisted. We envisagethe model developed hereinhopefully acting as a blueprint/model foraccurately reporting multi/extensive drug resistance; hence keeping stakeholders abreast of trends in susceptibility/resistance;towards managing the threat ofantimicrobial resistance.

Keywords: Antibiogram, Multidrug Resistance, Multiple Antibiotic Non-Susceptibility Index, Multiple Antibiotic Resistance Index

1. Introduction

Antimicrobial Resistance is one of the major problems threatening humanity (Abdullahi, 2019). With concomitant scientific and technological achievements, increases in the global consumption of antibiotics have been reported, with an attendant manifestation of microbes capable of fighting against these drugs, especially during the last 40 years; and the highest effect of this dramatic change is being witnessed in sub-Saharan Africa (Kariuki and Dougan, 2014). As the Charles Clift Centre for Global Health Security (CCCGHS, 2019) puts it, if the status quo is maintained, by 2050, the yearly global mortality burden due to antimicrobial resistance will equal 10 million people.
The most common type of manifestation of antimicrobial resistance is antibacterial resistance, where the bacteria resist antibiotics or antimicrobial agents to which they were susceptible before (Wiley *et al.*, 2013). The mechanisms through which this enables are many. For instance, some bacteria, such as the salmonellae, produce enzymes that cleave the Antibiotic that attacks them, subsequently inactivating it, such as the beta-lactamases (Abdullahi and Abdulkadir, 2019). Other methods may be molecular, involving resistance genes transferred through mobile genetic elements, such as plasmids and integrons/transposons. These genes are highly promiscuous, being transferred from one bacterium to another, with ease, via mechanisms including plasmid-mediated conjugation, transformation, and or transduction (Adesoji*et al.*, 2016).

The development of antimicrobial resistance is facilitated by many factors, including administering antibiotics in animal foods (Abdullahi and Abdulkadir, 2019). Other factors include intrinsic resistance (e.g., absence of the drug's target site); destitution, lack of education, and lack of accessibility to qualitative healthcare, which make certain individuals habour resistant strains of pathogens which may be transferable to other people, or the environment; poor quality of the drugs being consumed by the individuals; self-medication, failure to religiously adhere to therapeutic regimens; under and overdosage; lack of financial means of purchasing high-quality drugs; consumption of drugs which have expired or are improperly stored and prescription of wrong antibiotics for individuals (Riko*et al.*, 2020).

The presence of bacteria that can resist more than one Antibiotic, known as multidrug-resistant organisms (MDROs), further exacerbates this challenge. It may be difficult to find appropriate drugs to be used in therapy. At times, no active antibiotic can be found to efficiently cure the disease (Magiorakos*et al.*, 2011). These MDROs are found among both Gram-positive and Gram-negative bacteria, and infections with them can lead to therapeutic failure, which can escalate to mortalities (Cassir*et al.*, 2014). Thus, tackling them is a quintessential task. However, it requires epidemiological surveillance studies that traverse diverse healthcare settings and countries (Doi*et al.*, 2009).
The importance of lab-based surveillance in tackling Antibiotic Resistance is underscored by the WHO's 2015 Global Strategy for Containment of Antibiotic Resistance. They termed it a "fundamental priority" in evolving techniques of limiting antimicrobial resistance (Nasir *et al.*, 2013). Nonetheless, the lack of sufficient, qualitative data on surveillance of susceptibility/resistance patterns of microbes from developing countries undermines the global push towards fighting infections. Absence of communication of findings from one lab to another, with provisions for collaborative surveillance, hinders observers from being up-to-date regarding emerging patterns of antimicrobial resistance, identification, characterization, and control of antibiotic threats evaluation of national statuses of fights against antibiotic Resistance (CDC, 2009).

Among the many benefits derivable from studies of such kind include official documentation of research procedures and rapid dissemination of results to a surveillance database network, where it exists, in and outsides states, and contrasting results from similar studies conducted in different laboratories. These are key to understanding and combatting Antibiotic Resistance (WHO, 2015).

One of the key indicators of multidrug resistance in bacteria was the MAR Index, which was proposed by Krumperman (1983), who defined the MAR Index thus:

"The MAR index, as when applied to a single isolate, is defined as a/b, where a represents the number of antibiotics to which the isolate was resistant, and b represents the number of antibiotics to which the isolate was exposed".

Krumperman further defined MAR indexing, when there are many isolates identified froma sample,as *"a/(b*c), where a is the aggregate antibiotic resistance score of all isolates from the sample, b is the number of antibiotics, and c is the number of isolates from the sample".*For instance, 30 isolates having an aggregate antibiotic score of 240 taken from a sample wouldhave the sample's MAR index as 240/(12 x 30), i.e.0.66 (Krumperman, 1983).

Since its introduction, the MAR Index has witnessed numerous applications in the Nigerian research sphere. A selection of studies from each of Nigeria's geo-opolitical zones, where MARI was used in determination of antimicrobial resistance is presented in Appendix III.
The MARI had been the subject of many interpretations. There are usually two major approaches: first, organisms aving a MARI of 0.2 and above as though to be growing in an environment contaminated with multiple antibiotics (Tambekar*et al.*, 2006; Oli*et al.*, 2013) and second, MARI values of 0.3 and above indicate multidrug resistance (See, for instance, most of the studies in appendix III).

However, there is a more accurate interpretation of MARI values. This is based on the International Experts report by scientists from the Centres for Disease Control and Prevention, USA and the European Centres for Disease Prevention and Control, them in turn dependent on data fromClinical Laboratory Standards Institute (CLSI), the European Committee on Antimicrobial Susceptibility Testing (EUCAST) andthe United States Food and Drug Administration (FDA). They defined multi/extensive/pan-drug resistance as follows, respectively:

"MDR … defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR … defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one

or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories" (Magiorakos*et al.*, 2011). In other words, MARI values of 0.3 and above, where at least three resistances were manifested against different classes of antibiotics constitute multidrug resistance. Moreover, non-susceptibility includes both 'resistant' and 'intermediate' antimicrobial susceptibility results. For details and examples on the applications of these definitions in the current study, consult sections 2.7 and appendixes I-II.

The relationship between all bacteria, multidrug resistant bacteria, extensively drug-resistant bacteria and pan drug-resistant bacteria is given in figure 1 below:

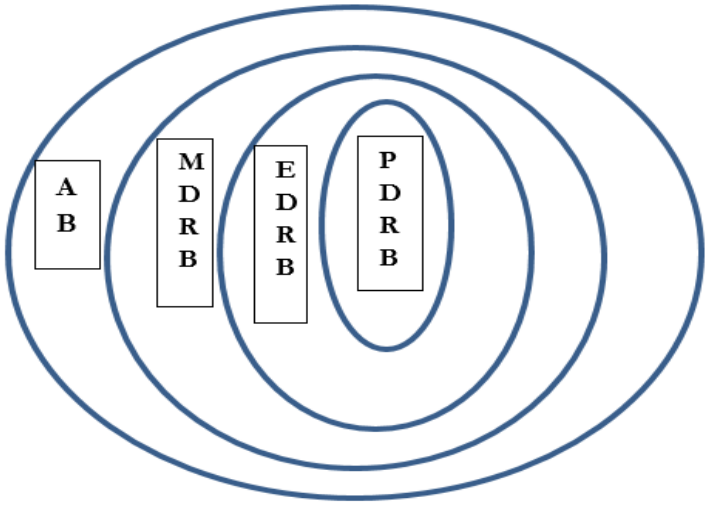

Figure 1: Relationship between All Bacteria (AB), Multidrug Resistant Bacteria (MDRB), Extensively Drug-Resistant Bacteria (EDRB) and Pan Drug Resistant Bacteria. MDRB is a subset of AB; EDRB is a subset of MDRB and PDRB is a subset of EDRB.

There had been a few studies in Nigeria which had attempted to utilise the criteria from Magiorakos*et al.* for classifying antibacterial resistance, with varying degrees of success and different methodological approaches (To the best of our research we have come across only two: Otokunefor*et al.*, 2018 and Bala*et al.*, 2019). Another study by Aworh*et al.* used the same methodology for determining multidrug resistance, but did not cite Magiorakos et al. as their source; furthermore, there was no mention of procedural methods for determining extensively-drug resistant (XDR) or pan-drug resistant (PDR) organisms.

A review of literature conducted across Nigeria's six geopolitical zones (see appendix III), showed that there is a wide inconsistency in terms of standards for reporting and interpreting both Sensitive, Intermediate and Resistanceand MARI values. Most researchers do not follow any laid guideline at all, with only a few following the rule of 'multidrug resistance = resistance to antibiotics in three or more classes'. With the new information supplied in the CDC/ECDCguidelines, in turn based on CLSI, EUCAST and FDA data, the use of the existent protocols for reporting results of surveillance in the Nigerian contexts becomes redundant; as such there is the need for a revisit to the protocols for reporting data or results. As such, the research gap seeking to be bridged, i.e. the adaption of the CDC/ECDC guidelines in Nigerian contexts, with regard to the commonest Nigerian antibiotics in use, and other less common ones, to enable the streamlining and development of a uniform model for reporting results of antibiotic resistance surveillance studies which will take into consideration the latest pieces of information from the CDC/ECDC guidelines, together with theCLSI, EUCAST and FDAinputs to allow their easy interpretation at a glance, and facilitate seamless transfer of information from researchers and stakeholders to partner agencies, authorities, policy makers and the general public. This is what this research aims to achieve.

Furthermore, it is envisaged that an endeavour of this kind will provide a platform for uniformly grading the antibiograms of various organisms, leading to the production of reliable data which can be used in monitoring antimicrobial resistance and susceptibility patterns in various locales (Magriakos*et al.*, 2011; Carmelli*et al.*, 2010; Jones and Masterton, 2001). This is especially important in laboratory and clinical settings, public health and epidemiology studies, and it is hoped that effective application of the model will help provide information in a

condensed form, as a useful aid to stakeholders in antimicrobial resistance-related issues, including the government and policy makers, in designing strategies to combat multidrug resistance.

1.1 Study objective

The study's objective is to carry out a surveillance study on the most frequently isolated gram-negative bacteria in the Microbiology Laboratory of Umaru Musa 'Yar' adua University, Katsina, and test the presence or absence of multidrug/extensively drug-resistant bacteria among the isolates, using a robust model developed for classifying bacteria into multi/extensively-drug resistant in the Nigerian context, adopted from an International Experts' Group's Guidance, i.e.Magriakoris*et al.* (2011).

1.2 Hypothesis

The study hypothesized the following hypothesis as a guide:

Null hypothesis: *Most commonly identified gram-negative bacteria from Katsina Metropolis, Nigeria, do not includemultidrug resistant strains.*

2. Methodology

2.1 Study Design

The study was a cross-sectional surveillance study on the presence of multidrug resistant bacteria from various food, environmental and clinical samples collected from Katsina Metropolis, Katsina State, Nigeria, from 2018-2020. The most frequently isolated gram-negative bacteria alone are considered for the study to ensure the data's representativeness. Likewise, only one genus was chosen from each sample from where thestrains of the bacteria were isolated.

2.2 Sourcing Test Organisms: Isolation and Identification

The organisms involved in the study were sourced from the culture collection at the Microbiology Laboratory, Department of Microbiology, Umaru Musa 'Yar' adua University, Katsina. Initially, the gram-negative bacteria were sourced from various sources to ensure that a cross-sectional approach was taken to do the research and evaluate whether an isolation environment can affect the presence/absence of multidrug resistance. Such sources include food samples (awara/tofu/soybean cake), medical samples (sputum sample, urine sample), and environmental samples (fomites/door handles, insect vectors/house fly & cockroach) (Table 1). These organisms were selected from various samples to test the overall presence of resistance in multiple, diverse samples from the study area (Liu *et al.*, 2013; Kayode*et al.*, 2020).

Standard protocols were followed for isolation and identification, as guided by Kabir*et al.* (2020). Briefly, the spread plate technique (Wiley *et al.*, 2013) was used for isolation, and the identification followed the three-tiered approach of Darma*et al.* (2019). First, bacterial cultures were subjected to colonial morphology identification; next, they were identified based on cellular morphology, and finally, they were subjected to a catalog of biochemical tests, after which identification keys and tables (Barrow and Feltham, 2004) were used to aid the identification of the bacteria.

1.3 Maintenance/Preservation of Identified Cultures

Upon being isolated, the bacterial cultures were maintained and preserved using two methods: agar slant preservation, with continuous, periodic (quarterly) re-transfer onto fresh slants (Solunke, 2019) and storage in glycerol stocks (Howard, 1956).

1.4 Reviving of Isolates

The isolates were revived as modified from the ATCC (2015) and Garrity (2012). Briefly, the preserved slants/glycerol stock cultures were swabbed with a sterilized wire loop and inoculated into a freshly prepared nutrient broth medium. The organized were enriched for 48 hours before being plated on nutrient agar, purified via

successive plate transfer technique (modified from Darma*et al.*, 2019), and re-subjected to the identification protocols outlined above ensure the purity of the cultures and maintenance of their biochemical properties/identity.

1.5 Antibiogram Determination

The antibiogram of each identified bacterium was determined using the protocols of Kayode*et al.* (2020), with little modifications. Briefly, 24 hours old purified cultures of the test organisms grown on nutrient agar were used in preparing standardized inoculums matching the 0.5 McFarland standards, equivalent to 1.5 x 10^8 cells/ml. The agar disc diffusion method was used for susceptibility testing using ten commonly used antibiotics on Mueller-Hinton Agar. The antibiotics used and their concentrations are: Augmentin/AU (30µg), Septrin/SXT (30µg), Streptomycin/S (30µg), Ofloxacin/Tarivid/OFX (10µg), Ciprofloxacin/CPX (10µg) Pefloxacin/Reflacin/PEF (10µg), Ampicillin/PN (30µg), Gentamicin/CN (10µg), Ceprox/CEP (10µg) and Nalidixic Acid/NA (3mg). The results were interpreted after 24 hours of incubation at 37^{o}C, using the guidelines of Clinical and Laboratory Standards Institute (2014), CLSIFDA (2013), in turn, adapted from CLSI (2013) and Bala*et al.* (2019).

1.6 Evaluation of MARI

The multiple antibiotic resistance index of the antibiotics was determined using the following formula:

MARI = Number of Antibiotics the Bacterium Resists
Total Number of Tested Antibiotics

1.7 Development of Protocol for the Determination of Multidrug/Extensively Drug-Resistant Bacteria

We develop a model for defining a multidrug/extensively drug-resistant bacterium based on definitions adopted from Magiorakos*et al.* (2012) viz: A bacterium is considered multidrug resistant if it is non-susceptible (resistant or intermediate) to three or more antibiotics that belong to at least three different classes. It is considered extensively drug-resistant if it resists/shows intermediacy to drugs from all but two or one among the different classes/types of antibiotics tested against it.

2.7.1 The Multiple Antibiotic Non-Susceptibility Index (MANSI) and theClass Non-Susceptibility Index (CNSI)

From here, we deem it fit to develop a new model, based on modifications of the Multiple Antibiotic Resistance Index-based approach. We hence defined two new terminologies. Firstly, we define the Multiple Antibiotic Non-Susceptibility Index/MANSI as: '***the ratio of antibiotics the bacterium is not-susceptible to (resistant or intermediate) to that of the total number of tested antibiotics***', as represented by equation 1 below:

MANSI = Number of Antibiotics the Bacterium Resists/is Intermediately Susceptible to
Total Number of Tested Antibiotics ……. (1)

As such, a bacterium, resistant to two different classes of antibiotics and showing intermediacy to another antibiotic in the same class will have a MANSI of 0.3, but is not multidrug resistant, however, the same bacterium, when it exhibits intermediacy to another antibiotic in a separate class of antibiotics will have a MANSI value of 0.3 and will be regarded as multidrug resistant.

Finally, we define the Class Non-Susceptibility Index/CNSI as: 'the ***total number of antibiotic classes against which the bacterium shows resistance to at least one antibiotic member***'. As such, the CNSI becomes an easy tool for detecting multidrug resistance. If a bacterium has a CNSI of 3 and above, it is multidrug resistant. If the CNSI differs from the total number of antibiotics tested in a study by just a factor of two or one, then then bacterium is extensively-drug resistant, (e.g. a CLSI of 6/7 when there are 7 or 6/8 when there are 8 classes of antibiotics in the whole battery of antimicrobials used inthe antibiogram), and if the CNSI is equivalent to the number of different classes of antibiotics tested, and, additionally, the MANSI values is exactly 1.0, then the bacterium is pan-drug resistant, e.g. a CNSI of 8/8 when 8 different classes of antibiotics were tested , plus a MANSI of 1.0(For examples of the applicability of the definitions, consult appendixes I-II below). To ease knowing

the number of antibiotic classes being dealt with, the result remains expressed as a fraction, as shown in equation 2 below:

$$\text{CNSI} = \frac{\text{Number of Antibiotic Classes the Bacterium Resists/is Intermediately Susceptible to}}{\text{Total Number of Antibiotic Classes Tested}} \quad \ldots\ldots\ldots (2)$$

(Where the result remains expressed as a fraction).
We utilised commonly incorporated antibiotics in commercially prepared gram-negative antibiotic sensitivity disks sold in Nigeria for the development of the model. Furthermore, we conducted a cursory search of literature for other antibiotics that had been reported in researches on multidrug resistance in Nigeria, aside from those available in the commercially prepared antibiotic disks. For the determination of the class of an antibiotic, *Jawetz, Melnick and Adelbergs's Medical Microbiology* (Brooks *et al.*, 2013) was consulted, in addition to the blueprint designed by an Expert Group involving ECDC and CDC; relying on data from CLSI, EUCAST and FDA (Magiorakos*et al.*, 2011) and the Categorisation of Antibiotics Report by the European Medicines Agency (EMA, 2020).

1.8 Data Analysis

The data obtained were analyzed using Microsoft Office Excel Data Analysis ToolPak (2019 version). A pie chart was constructed to represent some part of the data, standard deviations were calculated, and the Analysis of Variance (ANOVA)'s p and F statistics were used in elucidating significant differences between compared groups, or lack thereof (Berk and Carey, 2010).

2. Results

The model developed for determining susceptibility or resistance was adopted from Magiorakos*et al.* (2011). The fully developed model is attached in appendixes I and II.The two appendixes differ,whereas in developing Appendix I,the commonest antibioticsreported in multidrug resistance assays for both gram positive and gram negative bacteria using commercially prepared antibiotic disks were considered, including broad and narrow spectrum antibiotics. These antibiotics were the most commonly employed ones in the UMYU Microbiology Lab for antimicrobial susceptibility testing during the study period (2018-2020), likewise in hospitals, medical diagnostic centres/laboratories, etc. within Katsina. These antibiotics were also reported in previous studies across Nigeria, for instance Mustapha and Imir (2019). In the second appendix, a fairly thorough literaturesearch for other antibioticsthat had been reported by other researchers carrying out multidrug resistance studies in Nigeria(2015-2020), apart from the commonest in use stated in appendix I were conducted,and the results modelled after the first appendix.Specifically, the literatures consulted were stated as appendix III. In the most commonly used antibiotics model presented in Appendix I, 17 antibiotics, belonging to 11 different classes, were included. In all, 37 different antibiotics, belonging to some 22 different classes, were incorporated in both models.

The sources from where the test organisms for the study to check the m, delwere isolated are presented in Table 1 below. As the aim was to select bacteria from diverse locations, only one bacterium, the most frequently occurring, was chosen from each source.

Table 1: Sources of the selected gram-negative bacteria used in the study

S/No	Bacterium	Source
1	*Enterobacteraerogenes*	Fomite sample
2	*Escherichia coli*	Food sample
3	*Klebsiellapneumoniae*	Sputum sample
4	*Pseudomonas aeruginosa*	Urine sample
5	*Shigelladysentriae*	Insect Vector (Cockroach)

The bacteria's distribution in terms of their relative frequencies was represented in the pie chart below (Figure 2). The percentages given represent the overall likelihood of the bacterium being isolated, regardless of the sample. The percentages were obtained based on isolation and identification data at the laboratory. Thus, *Escherichiacoli* (91.67%) is isolated more frequently than *Enterobacteraerogenes* (25%).

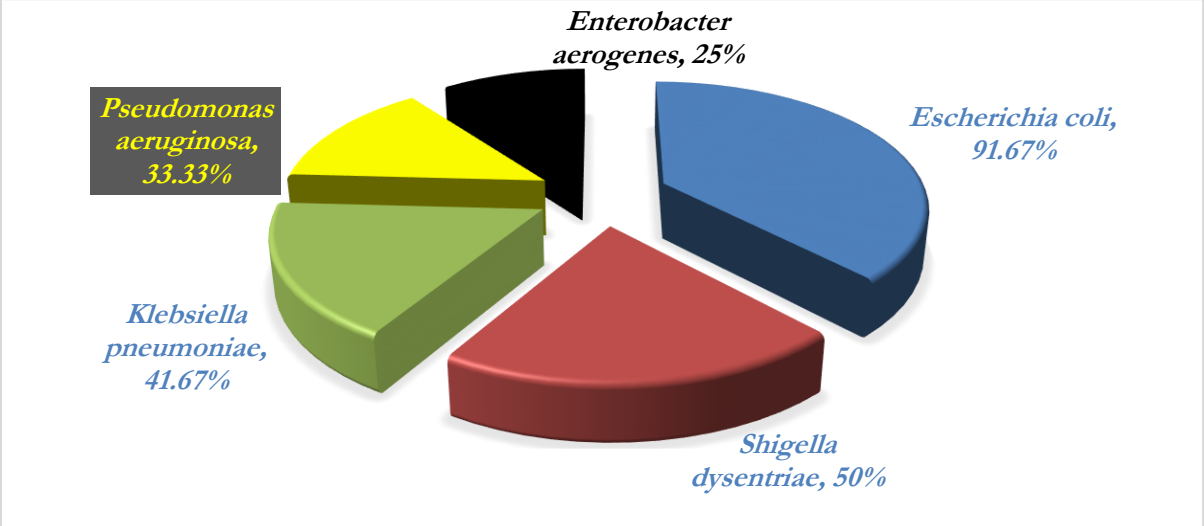

Figure 2: Relative Percentages (out of 100%) of the Frequency of Isolation of some Gram-Negative Bacteria from various Samples in UMYU Microbiology Lab, Katsina, Nigeria. Each percentage frequency represents tendency to isolate the bacterium from a given sample. Thus, roughly, 9 out of 10 samples habour *E. coli*, 4 habour *Klebsiella*, etc.

Regarding the antibiogram of the individual bacteria, *E. aerogenes* was resistant to half of the tested antibiotics and susceptible to the other half. It was found to be most vulnerable to Pefloxacin or Reflacin, with a zone of inhibition of 29.5±0.71 mm. The most resisted antibiotics were ceporex/cephalexin and nalidixic acid, which elicited the formation of no zone at all (Table 2).

Table 2: Antibiogram of *Enterobacteraerogenes*

S/No	Antibiotic (Abbreviation)	Disc Potency	Average Zone of It nhibition Generated (mm±S.D.)	Interpretation*
1	Ampicillin (P.N.)	30µg	12±1.41	Resistant
2	Augmentin (AU)	30µg	22.5±2.12	Susceptible
3	Ceporex/Cephalexin (CEP)	10µg	-	Resistant
4	Ciprofloxacin (CPX)	10µg	14±0.00	Resistant
5	Gentamicin (C.N.)	10µg	12±1.41	Resistant
6	Nalidixic Acid (NA)	3mg	-	Resistant
7	Ofloxacin/Tarivid (OFX)	10µg	24±2.83	Susceptible
8	Pefloxacin/Reflacine (PEF)	10µg	29.5±0.71	Susceptible
9	Septrin/Cotrimoxazole (SXT)	30µg	23.5±0.71	Susceptible
10	Streptomycin (S)	30µg	22.5±2.12	Susceptible

Key: S.D. = Standard Deviation, - = No zone of inhibition obtained and * = Interpretation of the zone of inhibition generated was based on the interpretative chart in appendix IV.

Escherichiacoli, on the other hand, was susceptible to all the antibiotics tested, albeit with varying zones of inhibition, which ranged from 34.5±6.36 for ciprofloxacin to 19.5±0.71 for both ampicillin and ceporex/cephalexin (Table 3). Regarding *K. pneumoniae,* resistance was obtained in 3 out of the ten antibiotics tested, with augmentin showing no zone of inhibition at all. An intermediate result was obtained for nalidixic acid and ciprofloxacin. The bacteria were susceptible to all remaining six antibiotics, with the highest zone being obtained in Streptomycin, with a zone of 31±0.00 mm. (Table 4).

Table 3: Antibiogram of *Escherichia coli*

S/No	Antibiotic (Abbreviation)	Disc Potency	Average Zone of Inhibition Generated (mm±S.D.)	Interpretation*
1	Ampicillin (P.N.)	30µg	19.5±0.71	Susceptible
2	Augmentin (A.U.)	30µg	23±1.41	Susceptible
3	Ceporex/Cephalexin (CEP)	10µg	19.5±0.71	Susceptible
4	Ciprofloxacin (CPX)	10µg	34.5±6.36	Susceptible
5	Gentamicin (C.N.)	10µg	26±4.26	Susceptible
6	Nalidixic Acid (NA)	3mg	25.5±2.12	Susceptible
7	Ofloxacin/Tarivid (OFX)	10µg	26.5±4.95	Susceptible
8	Pefloxacin/Reflacine (PEF)	10µg	26.5±2.12	Susceptible
9	Septrin/Cotrimoxazole (SXT)	30µg	22.5±2.12	Susceptible
10	Streptomycin (S)	30µg	28±2.83	Susceptible

Key: S.D. = Standard Deviation, * = Interpretation of the zone of inhibition generated was based on the interpretative chart in appendix IV.

Table 4: Antibiogram of *Klebsiellapneumoniae*

S/No	Antibiotic (Abbreviation)	Disc Potency	Average Zone of Inhibition Generated (mm±S.D.)	Interpretation*
1	Ampicillin (P.N.)	30µg	21.5±3.54	Susceptible
2	Augmentin (AU)	30µg	-	Resistant
3	Ceporex/Cephalexin (CEP)	10µg	11.5±0.71	Resistant
4	Ciprofloxacin (CPX)	10µg	19.5±0.71	Intermediate
5	Gentamicin (C.N.)	10µg	19±1.41	Susceptible
6	Nalidixic Acid (NA)	3mg	16.5±0.71	Intermediate
7	Ofloxacin/Tarivid (OFX)	10µg	17.5±0.71	Susceptible
8	Pefloxacin/Reflacine (PEF)	10µg	21.5±0.71	Resistant
9	Septrin/Cotrimoxazole (SXT)	30µg	19.5±0.71	Susceptible
10	Streptomycin (S)	30µg	31±0.00	Susceptible

Key: S.D. = Standard Deviation, - = No zone of inhibition obtained and * = Interpretation of the zone of inhibition generated was based on the interpretative chart in appendix IV.

Moreover, the bacterium *P. aeruginosa* showed intermediacy to three antibiotics and susceptibility to five. The highest zone was obtained for gentamicin (31.5±3.54 mm). The bacterium was resistant to ceporex or cephalexin and pefloxacin/reflacin, with no inhibition zone produced by the former antibiotic(Table 5).

Table 5: Antibiogram of *Pseudomonas aeruginosa*

S/No	Antibiotic (Abbreviation)	Disc Potency	Average Zone of Inhibition Generated (mm±S.D.)	Interpretation*
1	Ampicillin (P.N.)	30µg	16±0.00	Intermediate
2	Augmentin (AU)	30µg	17.5±2.12	Susceptible
3	Ceporex/Cephalexin (CEP)	10µg	-	Resistant
4	Ciprofloxacin (CPX)	10µg	19.5±0.71	Intermediate
5	Gentamicin (CN)	10µg	31.5±3.54	Susceptible
6	Nalidixic Acid (NA)	3mg	15.5±3.54	Intermediate
7	Ofloxacin/Tarivid (OFX)	10µg	21±1.41	Susceptible
8	Pefloxacin/Reflacine (PEF)	10µg	14.5±3.54	Resistant
9	Septrin/Cotrimoxazole (SXT)	30µg	27±1.41	Susceptible
10	Streptomycin (S)	30µg	28±2.83	Susceptible

Key: S.D. = Standard Deviation, - = No zone of inhibition obtained and * = Interpretation of the zone of inhibition generated was based on the interpretative chart in appendix IV.
For *S. dysentiae,* resistance was manifested against five antibiotics, with no inhibition zone being produced by ceporex/cephalexin, nalidixic acid, and pefloxacin/reflacine. Furthermore, the bacterium showed intermediacy to one and susceptibility to four of the antibiotics tested, i.e., ampicillin (19±2.83 mm), gentamicin (20±1.41 mm), septrin/clotrimoxazole (16.5±0.71 mm) and streptomycin (18±1.41) (Table 6).

Table 6: Antibiogram of *Shigelladysentriae*

S/No	Antibiotic (Abbreviation)	Disc Potency	Average Zone of Inhibition Generated (mm±S.D.)	Interpretation*
1	Ampicillin (P.N.)	30µg	19±2.83	Susceptible
2	Augmentin (AU)	30µg	10.5±0.71	Resistant
3	Ceporex/Cephalexin (CEP)	10µg	-	Resistant
4	Ciprofloxacin (CPX)	10µg	12±0.00	Resistant
5	Gentamicin (C.N.)	10µg	20±1.41	Susceptible
6	Nalidixic Acid (NA)	3mg	-	Resistant
7	Ofloxacin/Tarivid (OFX)	10µg	14.5±2.12	Intermediate
8	Pefloxacin/Reflacine (PEF)	10µg	-	Resistant
9	Septrin/Cotrimoxazole (SXT)	30µg	16.5±0.71	Susceptible
10	Streptomycin (S)	30µg	18±1.41	Susceptible

Key: S.D. = Standard Deviation, - = No zone of inhibition obtained and * = Interpretation of the area of inhibition generated was based on the interpretative chart in appendix IV.

The MARI determination results showed that one organism have MARI index below 0.2, i.e., *Escherichiacoli,* with a MARI of 0.0, while the rest have MARI indexes equal to or above 0.2, i.e.*Pseudomonasaeruginosa*(0.2), 0.3 (*Klebsiellapneumoniae*), 0.4 (*Shigelladysentriae*) and 0.5 (*Enterobacteraerogenes*). According to the definition given before, all three are multidrug resistant as they resist drugs from at least three different classes.

However, when the Multiple Antibiotic Non-Susceptibility Index was considered, which is more encompassing than MARI, as where both intermediacy and resistance to the antibiotics are factored in, it becomes apparent that four bacteria have a MANSI of ≥ 0.5, with *E. aerogenes, K. pneumoniae* and *P. aruginosa* having a MANSI of 0.5 each, and *S. dysentraiae* having a MANSI of 0.6. As such, based on the Class Non-Susceptibility Index, we can see that the four bacteria mentioned above have CNSI values that place them into the multidrug resistant category: *K. pneumoniae, P. aeruginosa* and *S. dysentriae* all show non-susceptibility to antibiotics from four different classes, for a CNSI of 4/8,while *E. aeruginosa*, with a CNSI of 5/8, showed non-susceptibility to antibiotics from five different classes. However, as the total number of antibiotic classes was 8, and no bacterium has a CNSI of at least 6/8, we conclude that none of the tested bacteria were extensively drug resistant. *E. coli*, with MANSI and CNSI values of 0.0 and 0, respectively, was neither multidrug nor extensively drug resistant.

From the table, it can be seen that the cephalosporins (ceporex, 80% resistance) were the most resisted antibiotics, followed by the fluoroquinolone pefloxacin/reflacine (60% resistance) and beta-lactam + beta-lactamase inhibitor (Augmentin), non-fluorinated quinolone (nalidixic acid) and ciprofloxacin, which all have resistance values of 40%. The most effective antibiotics were the sulfonamide + Trimethoprim, i.e., septrin, with 100% susceptibility, followed by the aminoglycosides: gentamicin and Streptomycin (80% susceptibility each) and ofloxacin/derived, with 80% susceptibility too, which is from the quinolone family (Table 7).

As such, an ***alternate hypothesis, H_A***, must be developed for this study, which states that: *multidrug resistant, commonly isolated gram negative bacteria exist in samples originating from medical, environmental and food sources.*

5 Discussion

The study isolated and identified five bacteria: *Enterobacteraerogenes, Escherichia coli, Klebsiellapneumoniae, Pseudomonas aeruginosa,* and *Shigelladysentriae*. Except for *P. aeruginosa*, the remaining species are all *Enterobacteriaceae* members. Their presence, from the sources described, i.e., fomites, food, sputum, and insect vectors, is not surprising but is indicative of potential contamination of the originals with the fecal-related matter (Kabir*et al*., 2020) except for the

sputum sample, as *K. pneumoniae* can readily be found autochthonously in the lungs (Brooks *et al.*, 2013). *P. aeruginosa*, on the other hand, is commonly associated with urinary tract infections (Kolawale*et al.*, 2009; Khorvash*et al.*, 2009). These results highlight the need to ensure stricter standards for foods to prevent them from contamination by these organisms (Kabir*et al.*, 2020).

Table 7: Screening of Bacteria for Multiple Antibiotic Resistance based on Exhibition of Resistance to Multiple Classes of Antibiotics

Classes of Antibiotics	Antibiotics Tested within the Class	Susceptibility/Intermediacy/ Resistance of the Tested Bacterium to the Antibiotic					Percentage Effectiveness of the Antibiotic		
		E.a	***E.c***	***K.p***	***P.a***	***S.d***	**%S**	**%I**	**%R**
Aminoglycosides*	Gentamicin	R	S	S	S	S	80%	0%	20%
Cephalosporins (1st or 3rdGeneration)	Ceporex (Cephalexin or Ceftriaxone)	R	S	R	R	R	20%	0%	80%
	Ciprofloxacin	R	S	I	I	R	20%	40%	40%
Fluoroquinolones (1st Generation)	Ofloxacin/Tarivid	S	S	S	S	I	80%	20%	0%
	Pefloxacin/Reflacine	S	S	R	R	R	40%	0%	60%
Folate Pathway Inhibitors	Septrin/Co-trimoxazole/Trimethoprim + Sulfamethoxazole	S	S	S	S	S	100%	0%	0%
Non-Fluorinated Quinolone	Nalidixic Acid	R	S	I	I	R	20%	40%	40%
Penicillins	Ampicillin	R	S	S	I	S	60%	20%	20%
Penicillins + β-lactamase inhibitor	Augmentin/Amoxicillin + Clavulanic Acid	S	S	R	S	R	60%	0%	40%
Streptomycins	Streptomycin	S	S	S	S	S	100%	0%	0%
% of total antibiotics the bacterium shows intermediacyto		***NA***	***NA***	***20%***	***30%***	***10%***			
% of total antibiotics the bacterium is susceptible to		***50%***	***100%***	***50%***	***50%***	***40%***			
% of total antibiotics the bacterium is resistant to		***50%***	***0%***	***30%***	***20%***	***50%***	**Average Susceptibility %**		**Average Resistance %**
Multiple Antibiotic Resistance Index (MARI)		***(0.5)***	***(0.0)***	***(0.3)***	***(0.2)***	***(0.5)***	**= 58%**		**= 30%**
Multiple Antibiotic Non-Susceptibility Index (MANSI)		***0.5***	***0.0***	***0.5***	***0.5***	***0.6***	**Average MARI = 0.3**		
Class Non-Susceptibility Index (CNSI)		***5/8***	***0/8***	***4/8***	***4/8***	***4/8***	**Average MANSI = 0.42**		
Is the bacterium Multidrug Resistant?		***Yes***	***No***	***Yes***	***Yes***	***Yes***	**Average CNSI = 3.4/8**		
Is the bacterium Extensively Drug-Resistant?		***No***	***No***	***No***	***No***	***No***			

Key: *E.a = Enterobacteraerogenes, E.c. = Escherichia coli, K.p= Klebsiellapneumoniae, P.a = Pseudomonasaeruginosa*, S.d = *Shigelladysentriae*, S = Sensitive, I = Intermediate, R = Resistant, * = Streptomycin was not included in the aminoglycosides, and was assigned its own class, according to the protocol of Magiorakos*et al.* (2011), NA = Not Applicable.

The statistical analysis of the results obtained from this study conducted using One Way Analysis of Variance indicated that the inhibition zones elicited by the various antibiotics are significantly different at $p \leq 0.05$ ($p = 0.0025$; $F_{cal} = 4.30$, $F_{crit} = 2.36$). This indicates non-homogeneity in terms of the efficacy of particular antibioticsagainst the test bacteria. In the same vein, there is a significant difference in terms of the zones of inhibition produced by a single antibiotic against the different bacterial isolates tested ($p = 0.000081$, $F_{cal} = 11.32$, $F_{crit} = 3.01$). This showed varying susceptibility/resistance profiles of the bacteria. These two findings further underscore the need for continuous and meticulous surveillance of individual bacterial strains against heterogenous antibiotics to adequately elucidate their various antibiogram profiles, considering the statistically significant differences that exist between them. These differences can also be as a result of structural and genomic differences amongst the test bacteria.

As can be summarily glanced from the last column (percentage effectiveness of the antibiotic), the percentage of antibiotic resistance observed in this study was lower than in many previous studies. On average, the resistance values are 0%, 20%, 30%, 50%, and 50%, giving a pooled average resistance rate of 30%, corresponding to a MARI Index of 0.30. This is in contrast to the results obtained by Kayode*et al.* (2020). They reported a prevalence of MARI Indexes ranging from 0.7-1.0, with 60.7% of the isolates resisting a minimum of 5 out of the seven antibiotics tested, from samples collected at the LadokeAkintola University Teaching Hospital, Osogbo, Osun State. Likewise, the results contrast with those reported by Abdullahi and Abdulkadir (2019), amongst non-typhoidal salmonellae isolated from Lagos and Katsina's chicken samples, which showed MAR Indexes of 0.00-0.93, with an average MARI of 0.473. Moreover, Ehinmadu (2003) also reported high MAR indexes for bacteria isolated from urine samples from Zaria, Kaduna State. The high MARI indexes reported in these studies might be as a result of the source of the bacterial isolates, i.e. their origination from a medical setting.

Interpreting the results from another perspective, four out of the five bacteria tested (Corresponding to 80%) have MARI values equivalent to or exceeding 0.3. This corresponds to Bala*et al.* (2019)'s findings, who reported that eighty-three percent (83.3%) of their isolates were MDR and have MARI greater than 0.3.

The observance of high MARI values indicates a variety of things. Firstly, as stated by Ejikeugwu (2013), MARI can be harnessed as a tool for evaluating the dispersion of antimicrobial resistance in the environment. High MARI values suggest previous exposure of the bacterial isolates to multiple antibiotics (Bala*et al.*, 2009), culminating in them developing resistance to it (Ekwalor*et al.*, 2016). Moreover, as a rule of thumb, MARI values greater than 0.2 are thought to indicate antibiotic misuse in the environment the organisms are isolated from (Tambekar*et al.*, 2006; Oli*et al.*, 2013).

The MANSI and CNSI values appear to be more all-encompassing that the MARI values, going by the critera set by Magiorakos*et al.* (2011), and have a better chance of predicting multidrug resistance than the MARI values alone, as had been shown in Section 3above.

In this study, the multidrug resistances of the isolate from a clinical specimen, i.e., *Klebsiellapneumoniae* and *Shigelladysentriae*, which has the potential to cause debilitating infections, are worrisome, as may complicate therapy, considering that the antibiotics resisted by these organisms are most commonly prescribed against diseases of the respiratory and gastrointestinal tract (Kayode*et al.*, 2020).

Resistance was observed mostly against the cephalosporin Ceporex/cephalexin (80% resistance), the quinolones and fluorinated quinolones (perfloxacin, ciprofloxacin and nalidixic acid), and the beta-lactam + beta-lactamase inhibitor (augmentin/co-amoxiclav). Resistance to ceporex and nalidixic acid had been reported widely. Resistance to ceporex may not be unconnected with the abundant prescription of cephalosporins(Prakash and Saxena, 2013) while resistance to nalidixic acid might be associated with its historic use. Resistance topefloxacin and ciprofloxacin is a new phenomenon which we can attribute to its frequent prescription in the study area, especially in gram negative bacterial infections, which might lead to the occurrence of resistance to the isolates (Abdullahi, 2019).

Resistance to augmentin is a bit surprising, even though it has been reported previously (Abdullahi and Abdulkadir, 2019). The use of Amoxicillin-Clavulanic Acid complex, also known as co-amoxiclav or augmentin, is supposed to be more effective than using a beta-lactam antibiotic alone, as clavulanic acid is supposed to protect the beta-lactam ring at the nucleus of the structure from attacks by beta-lactamases. However, the large augmentin size ishypothesized to hamper its permeability and transfer into bacterial cells, thus reducing the concentration to levels below critical thresholds (Ekwewalor*et al.*, 2016).

The source of the resistance in the isolate from the fomite (door handle) might be from improper handling, as door handles may be frequently touched by the hand surcease, which might be in quick contact with various microbes. Insect vectors might come into contact with the excreta of animals in the environment, and previous researches had proved that 50-90% of drugs given to animals are expelled into the atmosphere in non-metabolized forms or in intermediate ways which may be inactive but can readily transform into the consequent active forms (Adesoji*et al.*, 2016).
The most effective Antibiotic in the tested antibiotics was septrin/co-trimoxazole/trimethoprim + sulfamethoxazole, to which all the tested organisms were 100% susceptible. This is similar to Abdullahi and Abdulkadir (2019) findings, who reported 87% susceptibility of their *Salmonella* isolates from Katsina and Lagos to septrin. The high susceptibility/efficacy of the antibiotic may be connected to the fact that its rate of prescription for patients in the study area is low, and or its mechanism of action, which involves the inhibition of syntheses of components of nucleic acids (e.g., purines, thymidines) and proteins (e.g., methionine) synthesis, because the sulfamethoxazole, a sulfonamide, competes with p-aminobenzoic acid, thus inhibiting that step during folate synthesis. On the other hand, Trimethoprim competitively inhibits dihydrofolate reductase, which is used to synthesize tetrahydrofolate, which is the biologically active form of folate (Masters, 2003).

Streptomycin also has a 100% efficacy rate. This is also attributable to at least two factors: its low prescription rate in the study area, and its mechanism of action (an aminoglycoside antibiotic which inhibits protein synthesis by blocking the 30S ribosomal subunit, blocking the initiation complex of mRNA + formyl methionine + tRNA, causing a misread in the codons sequence and ultimately disintegrating polysomes into monosomes) (Brooks *et al.*, 2013; Madingan*et al.*, 2019). A previous study from the study area had also reported high susceptibility (80%) to streptomycin (Abdullahi and Abdulkadir, 2019).

Amongst the multidrug resistant bacteria identified in this study, *Enterobacteraerogenes* and *Pseudomonasaeruginosa* both resist five out of the ten antibiotics tested:*E. aerogenes*resisted antibiotics from five different classes, while *P. aeruginosa* resisted antibiotics from four different classes. The other multidrug resistant bacterium was *K. pneumoniae*, which resisted antibiotics from three different classes. that are multidrug resistant, *Klebsiellapneumoniae*and *Shigelladysentriae*, resist antibiotics from three classes. The result of our finding is relatively comparable to that as reported by Kayode*et al.* (2020). They observed that 57.4% of their MDR isolates resisted at least 4 out of the five antibiotics groups that they included in their study. This fact highlights the rapid proliferation/dissemination and acquisition of antibiotic resistance genes, longitudinally, in the environment (Kummerer, 2003), considering the isolates' various sources.

Conclusively, this study submits that even though MARI remains important in antimicrobial susceptibility/resistance studies, information from new research reports and expert guidelines necessitate the reconsideration of the MARI as the only tool to be used in ascertaining multidrug resistant. As such a model based on improvements upon the MARI, such as the model developed herein, with the newly defined indexes of Multiple Antibiotic Non-Susceptibility Index (MANSI)/Class-Non-Susceptibility Index (CNSI), that can provide better, more accurate representations of the true picture of antimicrobial resistance indexes.

When the tool was evaluated in the study area, the prevalence of commonly isolated, gram negative, multidrug resistant bacteria was found to be 80%. Werecommend continued widespread testing of bacteria and fungi in the study area, and accurate, unbiased reporting of the results of such surveillance studies in a summarised, convenient, information-rich model such as the one developed in the current study. This is expected to bridge the literature gap on overall prevalence of multidrugresistance in Katsina, Nigeria, and elsewhere. Thismodel, and probably subsequently improved version(s);is/are envisaged to hopefully serve as a blueprint/model for accurately reporting multidrug resistance surveillance researches; hence keeping stakeholders abreast of trends in susceptibility/resistance; towards managing the menace of antimicrobial resistance.

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47. Appendixes

Appendix I: Model Developed for ReportingMultidrug Resistance Based on most commonly used Commercially Prepared Antibiotics (Hypothetical Results used both for Gram Positive and Gram-Negative Bacteria)

Classes of Antibiotics	Test Antibiotic(concentration)	Susceptibility/Intermediacy/ Resistance of the Microbe to the Antibiotic					Percentage Effectiveness of the Antibiotic		
		A	***B***	***C***	***D***	***E***	**%S**	**%I**	**%R**
Aminoglycosides	Gentamicin* (10µg)	I	S	R	S	R	40%	40%	20%
Amphenicols/Phenicols	Chloramphenicol+(30µg)	I	-	S	-	R	33.33 %	33.33 %	33.33%
Ansamycins	Rifampicin/Rifampin+(20µg)	S	-	S	-	R	66.66 %	0%	33.33%
Fluoroquinolones	Ciprofloxacin* (10µg)	I	I	R	I	S	20%	60%	20%
	Levofloxacin+(20µg)	S	-	I	-	R	33.33 %	33.33 %	33.33%
	Norfloxacin+(10µg)	S	-	R	-	S	66.66 %	0%	33.33%
	Ofloxacin/Tarivid- (10µg)	-	S	-	S	-	100%	0%	0%
	Pefloxacin/Reflacine- (10µg)	-	R	-	I	-	0%	50%	50%
	Sparfloxacin+(10µg)	S	-	R	-	S	66.66 %	0%	33.33%
Folate Pathway Inhibitors	Septrin/Co-trimoxazole/Trimethoprim + Sulfamethoxazole-(30µg)	-	R	-	S	-	50%	0%	50%
Macrolides	Erythromycin+(30µg)	S	-	S	-	R	66.66 %	0%	33.33%
Non-Extended Spectrum Cephalosporins (1st Generation)	Ceporex/Cephalexin- (10µg)	-	S	-	I	-	50%	50%	0%
Non-Fluorinated Quinolones	Nalidixic Acid- (30µg)	-	S	-	I	-	50%	50%	0%
Penicillins	Ampicillin- (30µg)	-	S	-	I	-	50%	50%	0%
	Amoxicillin+(20µg)	S	-	S	-	R	66.66 %	0%	33.33%
Penicillins + β-lactamase inhibitor	Ampiclox/Ampicillin + Cloxacillin+(20µg)	S	-	S	-	R	66.66 %	0%	33.33%
	Augmentin/Amoxicillin + Clavulanic Acid- (30µg)	-	S	-	I	-	50%	0%	50%
Streptomycins	Streptomycin- (30µg)	-	S	-	I	-	50%	50%	0%

% of total antibiotics the bacterium shows intermediacyto	*30 %*	*10 %*	*10%*	*70 %*	*0%*	
% of total antibiotics the bacterium is susceptible to	*70 %*	*70 %*	*50 %*	*30 %*	*30%*	Average Susceptibility = 51%
% of total antibiotics the bacterium is resistant to/ Multiple Antibiotic Resistance Index (MARI)	*0% (0.0)*	*20 % (0.2)*	*40 % (0.4)*	*0% (0.0)*	*70% (0.7)*	AverageResistance = 22.59%
Multiple Antibiotic Non-Susceptibility Index (MANSI)	*0.3*	*0.3*	*0.5*	*0.7*	*0.7*	Average MARI = 0.26
Class Non-Susceptibility Index (CNSI)	*3/7*	*3/8*	*2/7*	*6/8*	*7/7*	Average MANSI = 0.50
Is the bacterium Multidrug Resistant?	*Yes*	*Yes*	*No*	*Yes*	*Yes*	Average CNSI = 4.2/8
Is the bacterium Extensively Drug-Resistant?	*No*	*No*	*No*	*Yes*	*Yes*	

Key: * = broad spectrum antibiotic used against both gram-positive and gram-negative bacteria;+ = Narrow spectrum antibiotic normally used for gram positive bacteria; - = Narrow spectrum antibiotic normally used for gram negative bacteria; total number of antibiotics classes tested = 8 (gram-negative)/7 (gram-positive).

NB: From the model above, using CNSI as a criterion, it is clear that A is a gram-positive, multidrug resistant bacterium, B is a gram-negative, multidrug resistant bacterium, C is a gram-positive bacterium that is neither multi-drug resistant, nor extensively-drug resistant, D is a gram-negative, extensively drug-resistant bacterium and E is a gram-positive, extensively drug-resistant bacterium. These results would not have been obtained had MARI alone been used as the judgement criterion. Thus, A had a MARI of 0, yet is still multi-drug resistant, B had a MARI of 0.2, and is multi-drug resistant,C had a MARI of 0.5, and is neither multi nor extensively drug-resistant, D had a MARI of 0.0 and is extensively drug-resistant, and E had a MARI of 0.5, and is extensively drug-resistant as well.

AppendixII: Model Developed for Reporting Multidrug Resistance Based on other Antibiotics in Use in Multidrug Resistance Studies (Hypothetical Results used Both for Gram Positive and Gram-Negative Bacteria)

Classes of Antibiotics		Antibiotics Tested	Susceptibility/Intermediacy/ Resistance of the Microbe to the Antibiotic					Percentage Effectiveness of the Antibiotic		
			A	B	C	D	E	%S	%I	%R
Aminoglycosides		Kanamycin-	-	I	-	I	-	0%	100%	0%
Amphenicols/Phenicols		Florfenicol-	-	I	-	I	-	0%	100%	0%
Anti-staphylococcal penicillins/β-lactams		Oxacillin+(1µg)	R	-	S	-	S	66.67 %	0%	33.33 %
Carbapenems		Imipenem- (10µg)	-	S	-	I	-	50 %	50%	0%
Cephamycins		Cefoxitin+(30µg)	R	-	S	-	R	33.33 %	0%	66.67 %
Extended Spectrum Cephalosporins	1st Generation	Ceporex/Cephalexin	R	I	R	I	I	0%	60%	40%
		Cephalothin(30µg)	R	S	R	I	S	40 %	20%	40%

	2nd Generation	Cefuroxime (30µg)	R	I	R	I	R	0%	40%	60%
Extended Cephalosporins Spectrum		Cefixime(5µg)	R	R	R	I	R	0%	20%	80%
	3rd Generation	Ceftazidime(30µg)	R	S	R	I	I	20 %	40%	40%
		Ceftiofur-	-	S	-	I	S	66.67 %	33.33 %	0%
		Ceftriaxone (30µg)	R	S	R	I	S	40 %	20%	40%
		Cefotaxime	R	R	R	I	S	20 %	20%	60%
Glycopeptides		Vancomycin+(30µg)	R	-	S	-	I	33.33 %	33.33 %	33.33 %
Lincosamides		Lincomycin+	R	-	S	-	I	33.33 %	33.33 %	33.33 %
Nitrofurans		Nitrofurantoin (300µg)	R	I	S	I	I	20 %	60%	20%
Polymyxins		Colistin- (10µg)	-	R	-	I	-	0%	50%	50%
Sulfonamides		Sulfamethoxazole	R	R	S	I	R	20 %	20%	60%
Tetracyclines		Tetracycline- (30µg)	-	R	-	I	-	0%	50%	50%
		Oxytetracycline+	R	-	S	-	I	33.33 %	33.33 %	33.33 %

% of total antibiotics the bacterium shows intermediacyto	***0%***	***33.33 %***	***0%***	***100%***	***40%***	
% of total antibiotics the bacterium is susceptible to	***0%***	***33.33 %***	***50 %***	***0%***	***33.33 %***	**Average Susceptibility = 23.83%**
% of total antibiotics the bacterium is resistant to/	***100 % (1.0)***	***33.33 % (0.33)***	***50 % (0.5)***	***0% (0.0)***	***20% (0.2)***	**AverageResistance = 37%**
Multiple Antibiotic Resistance Index (MARI)						**Average MARI = 0.41**
Multiple Antibiotic Non-Susceptibility Index (MANSI)	***1.0***	***0.67***	***0.5***	***0.6***	***0.67***	**Average MANSI = 0.69**
Class Non-Susceptibility Index (CNSI)	***9/9***	***8/9***	***2/9***	***9/9***	***7/9***	**Average CNSI = 6.8/8**
Is the bacterium Multidrug Resistant?	***Yes***	***Yes***	***No***	***Yes***	***Yes***	
Is the bacterium Extensively Drug-Resistant?	***Yes***	***Yes***	***No***	***Yes***	***Yes***	
Is the bacterium Pan Drug-Resistant?	***Yes***	***No***	***No***	***Yes***	***No***	

Key: * = broad spectrum antibiotic used against both gram-positive and gram-negative bacteria; + = Narrow spectrum antibiotic normally used for gram positive bacteria; - = Narrow spectrum antibiotic normally used for gram negative bacteria; total number of antibiotics classes tested = 9 (for both gram-negative&gram-positive).

NB: From the model above, using CNSI as a criterion, it is clear that A is a gram-positive, multi, extensively and pan drug-resistantbacterum; B is a gram-negative, multi and extensively drug resistant bacterium; C is a gram-positive bacterium that is neither multi nor extensively drug resistant; D is a gram-negative, multi, extensively and pan drug-resistant bacterium and E is a gram-positive, multi and extensively drug-resistant bacterium.
Again, this illustrates the usefulness of CNSI over MARI, and, to a lesser extent, MANSI. Specifically, C has a MARI of 0.5, but is neither multi nor extensively drug-resistant, because the CNSI is 2/9, which is lower than the required (i.e. 3 and above) for multidrug resistance, and lower as well than the required (at least 7/9 and above) for extensively drug-resistant.

Appendix III:Selected LiteraturesShowing the use of MARI in Determining Antimicrobial Resistance from Researches Conducted in all the Geopolitical Zones of Nigeria. The Range of Antibiotics Used in the various Multidrug Resistance testswas Consulted for Designing the Model in Appendix II.

S/No	Description of Experiment	State(s)/Geopolitical Zone of the Study	Antibiotics used in the Study	Citation
1	Evaluating the multidrug resistance of 105 bacterialisolates from raw, treated and municipal water sources, 2010-2011	Osun, Oyo and Ondo/South-West	Ceftiofur(CEF); Chloramphenicol (C); Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Nalidixic Acid (N); Sulfamethoxazole (SU); Sulfamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)	Adesoji*et al.* (2015)
2	Antibiotic resistance profiles of five Gram negative bacterial species identified from 24 samples collected fromabattoir waste, processing waster and products	Katsina/North-West	Amoxicillin (25μg), cotrimoxazole (25μg), nitrofurantoin (300μg), gentamicin (10μg), nalidixic Acid (30μg), ofloxacin (30μg), augmentin (30μg) and tetracycline (30μg)	Adesoji*et al.* (2016)
3	Antibiogram study on 215 isolates belonging to ten different Gram positive and Gram negative bacteria isolated from urine samples	Anambra/South-East	Ceftazidime (CAZ) 30μg, Cefuroxime (CRX) 30μg, Gentamicin (GEN) 10μg, Cefixime (CXM) 5μg, Ofloxacin (OFL) 5 μg, Augmentin (AUG) 30μg, Nitrofurantoin (NIT) 30 μg, and Ciprofloxacin (CPR) 5μg	Ekwealor*et al.* (2016)
4	Antimicrobial resistance profile of 35 nonclinical Gram negative bacteria isolated from surface water, slaughter houses and hostels	Rivers/South-South	Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Cefixime (5 µg), Ofloxacin (5 µg), Augmentin (30 µg), Nitrofurantoin (300µg) and Ciprofloxacin (5 µg))	Otukenafor*et al.* (2018)
5	Multidrug resistance evaluation of 48 *E. coli* isolates from farm workers and poultry, 2018-2019	Abuja/North-Central	Ampicillin (10µg),amoxycillin/clavulanic acid (20/10µg), tetracycline (30µg), gentamicin (10µg), cefuroxime (30µg), streptomycin (10µg), chloramphenicol (30µg), nalidixic acid (30 µg), sulfamethoxazole-trimethoprim (10µg), cephalothin (30µg), nitrofurantoin (300µg),	Aworh*et al.* (2019)

			ceftriaxone (30µg), imipenem (10µg), colistin (10µg), ceftazidime (30µg) and cefotaxime (30µg)	
5	Evaluating the multidrug resistance profile of *Staphylococcusaureus* from 98 samples isolated from poultry and poultry workers in 12 poultry farms	Kano/North-West	Cefoxitin, Oxytetracycline, Ampicillin, Ciprofloxacillin, Vancomycin, Gentamicin, Chloramphenicol, Erythromycin, Augmentin and Oxacillin	Bala*et al.* (2019)
6	Multidrug resistance profile of 7 species of Gram negative bacteria identified from 77UTI patients attending a teaching hospital in the study area, 2017-2018	Osun/South-West	Cephalosporins (ceftazidime: CAZ-30µg, cefuroxime: CRX-30µg, ceftriaxone: CTR-30 µg), aminoglycoside (gentamycin: GEN-10µg), macrolides (erythromycin: ERY-5µg), fluoroquinolone(ofloxacin: OFL-5µg) and beta-lactam (amoxycillin–clavulanate:AUG-5µg)	Kayode*et al.* (2020)
7	Multidrug resistance profile of five Gram negative bacteria isolated from 96 cattle samples	Borno/North-East	OFX= Cefoxitin; PEF= Reflacine; CPX= Ciprofloxacin; AU= Augmentin; CN= Gentamycin; S= Streptomycin; CTX: Cefotaxime; NA= Nalidixic Acid; SXT= Septrin; PN= Ampicilin	Mustapha *et al.* (2020)

Appendix IV: Sensitivity Interpretative Chart (Gram Negative) (Used for Determining Sensitivity, Intermediacy and Resistance of the Selected Gram Negative Bacteria used in the Study, as Reported in Tables

S/No	Antibiotic	Disc Potency	Diameter of Average Zone of Inhibition (mm)		
			Susceptibility	Intermediacy	Resistance
1	Ampicillin (PN)	30µg	≥17	14-16	≤13
2	Augmentin (AU)	30µg	≥18	14-17	≤13
3	Ceporex/Cephalexin (CEP)*	10µg	≥ 14	-	≤14
	Ciprofloxacin	10µg	≥21	16-20	≤15
4	Ciprofloxacin-*P.a*** (CPX)****	5µg	≥26	-	≤26
5	Gentamicin (CN)	10µg	≥ 15	13-14	≤12
6	Nalidixic Acid (NA)	3mg	≥19	14-18	≤13
7	Ofloxacin/Tarivid (OFX)	10µg	≥ 16	13-15	≤12
8	Pefloxacin/Reflacine (PEF)	10µg	≥24	-	≤24
9	Septrin/Cotrimoxazole (SXT)	30µg	≥16	11-15	≤10
10	Streptomycin (S)	30µg	≥15	12-14	≤11

* = Values for Cephalexin taken from EUCAST (2018) cited in HiMedia (2020); ** = The Ciprofloxacin and values for *P. aeruginosa* were taken from EUCAST (2018) cited in HiMedia (2020); *** = Ciprofloxacin values for *P. aeruginosa* slightly differ from those of the members of *Enterobacteriaceae.*

Sources:CLSI (2018) and EUCAST (2018) cited in HiMedia (2020).