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IDENTIFICATION, ANTIMICROBIAL SUSCEPTIBILITY SCREENING AND ESBL-STATUS OF GRAM-NEGATIVE BACTERIA FROM HEALTHY HUMANS AND LIVESTOCK WASTE BY VITEK-2 AUTOMATED SYSTEM

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ABSTRACT

Bacteria in healthy body sites of humans and livestock waste may harbour antibiotic resistance and cause community-based opportunistic and resistant infections. The study profiled the antibiotic susceptibilities of resident bacteria in healthy humans and livestock waste. Gram-negative bacteria were isolated from 23 specimens including skin swabs (6), nasal swabs (4), urine (6), stool (3), chicken droppings (2) and cattle droppings (2). VITEK® 2 Automated System was used for identification, antimicrobial susceptibility and extended-spectrum β-lactamase (ESBL) production test of the isolates. Nineteen (19) Gram-negative bacteria belonging to five genera and six species were identified, including Escherichia coli (n=9) 47.4%, Klebsiella pneumoniae ssp pneumoniae (n=1) 11.1%, Enterobacter cloacae ssp dissolvens (n=1) 11.1%, Acinetobacter baumannii (n=3) 15.8%, Acinetobacter haemolyticus (n=1) 11.1%, and Providentia stuartii (n=4) 21.1%. The isolates showed highest resistances to Ampicillin (78.6%) and Piperacillin (63.2%) and high susceptibilities to Ertapenem, Amikacin, Ciprofloxacin and Levofloxacin (100%); Ceftazidime, Cefepime, Meropenem (94.7%); Cefoxitin (93.3%); Gentamicin and Tobramycin (73.7%). Multiple Antibiotic Resistance (MAR) index values above the critical limit of 0.2 were shown by 100% (4/4) of Providentia stuartii isolates, 75% (3/4) of Acinetobacter isolates and 33.3% (3/9) of E. coli isolates. All the isolates tested negative for ESBL production. The public health implication is that resident bacteria from healthy individuals harbouring antibiotic resistance may transmit these to other bacteria or cause resistant opportunistic infections difficult to treat. Resistant bacteria from livestock can be transmitted to humans through the food chain. Proper disposal or decontamination of human body secretions and livestock waste is necessary.

Keywords: Resident bacteria, antibiotic resistance, VITEK-2 System, *Providentia stuartii*, *Acinetobacter baumannii*.

INTRODUCTION

The easy availability and misuse of antibiotics in developing countries including Nigeria have promoted the development of resistance among bacterial strains, which is the cause of a global health crisis. Antibiotic resistance often borne on plasmids is easily spread during interactions among microorganisms. Human individuals host thousands of bacterial types, with different body sites having their own distinctive communities. The mouth, gut, respiratory tract, skin and vagina have varying diversities of resident microbiota (PLoS Human Microbiome Project, 2014).

Interactions with other microorganisms in such communities could potentially accelerate resistance evolution via horizontal transfer of resistance genes (Baumgartner *et al*., 2020).

Resident bacterial microbiota in humans do not cause disease in normal circumstances, but may cause infectious diseases in immune-compromised individuals, or have the potential to develop resistance during antibiotic therapy with broad spectrum antibiotics, an adaptation for survival which is achieved by various mechanisms including mutation, altered target, and acquisition of resistance plasmids through horizontal gene transfers (Colavecchio *et al*., 2017).

Human microbiomes are wide sources of antibiotic resistant genes (ARGs) and a potential reservoir for pathogenic bacteria to acquire more resistance genes. Human microbiomes are subject to various selective pressures, which can modulate the human resistome, such as antibiotic administration, diet, lifestyle and travel ((Sommer *et al*., 2009; Baron *et al*., 2018). Resistance can be intrinsic to commensal bacteria or can be acquired by transitional bacteria which need defenses in hostile areas (Rolain, 2013; Fancello *et al*., 2011). Bacteria can acquire resistance genes through bacteriophages, plasmids or transposons (Colavecchio *et al*., 2017; Li *et al*., 2019; Partridge *et al*., 2018).

Antibiotic-resistant strains can persist in the human host environment in the absence of selective pressure for a long time (Jernberg *et al*., 2010). Antibiotic resistance in human microbiota could be intrinsic (Rolain, 2013), or the results of selective pressure conferred by antibiotics that the gut microbes previously encountered and somehow managed to maintain in the gut (Cheng *et al*., 2012). Antibiotic resistance genes in the human gut bacteria can be exchanged among the gut microbiota and can also be transferred to other bacteria, even if the bacteria are just passing through the intestine. This situation represents a high risk with regard to the increased emergence of antibiotic-resistant human pathogenic bacteria (Jernberg *et al*., 2010).

In a functional screening of human gut microflora, Cheng *et al*., (2012) identified ARGs of diverse bacterial origin, including nonpathogenic species such as *Bifidobacterium longum*, as well as opportunistic pathogens such as *Streptococcus suis* and *Staphylococcus pseudintermedius* (Cheng *et al*., 2012). The potential for gene transfer in the human gut is very high due to the dense microbial population (Kazimierczak and Scott, 2007; Baumgartner *et al*., 2020), making it imperative to understand the role of these bacteria as donors disseminating the ARGs to other bacteria, especially the incoming pathogenic bacteria.

The gastrointestinal tract is constantly exposed to numerous bacteria from the environment through food, water, soil, other humans, or animals (Coates *et al*., 2019; Hillman *et al*., 2017). These incoming bacteria often harbor antimicrobial drug resistance genes, which can be transferred to the indigenous microbial communities through HGT, where they may enrich the pool of available antimicrobial resistance elements in the gut microbiota.

In a comparative cross-sectional study aimed at assessment the gut bacteria profile and antibiotic resistance pattern among psychotropic drug users and a control group of healthy people, the most frequently isolated bacteria from patients and apparently healthy controls were *E. coli*, which was 100 (80.6%) and 102 (84.3%) respectively. Among 100 *E. coli* isolated from patients, 99 (99.0%) were resistant to tetracycline and ampicillin each

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and 58 (58.0%) to cefotaxime. On the other hand, among bacteria isolated from control groups, 96 (94.1%), 57 (55.9%) and 50 (49.0%) of *E. coli* were found to be resistant to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole, respectively (Gashaw *et al*., 2021).

Opportunistic pathogens isolated from both community sources and hospital patients have been reported to show multiple drug resistance (MDR). In an Italian study, Temperoni *et al*., (2021), reported a high prevalence of antibiotic resistance among opportunistic pathogens isolated from patients with COVID-19 under mechanical ventilation. These included 105 Gram-negative bacteria (60.7%), of which *E. coli*, *A. baumannii* and *K. pneumoniae* were the most common species (29.5%, 15.2% and 11.4%, respectively). A large number of patients harbored MDR pathogens, especially those who had been exposed to antibiotics in the days before ICU admission. They found a high prevalence of carbapenem-resistant *A. baumannii* (CRAB) colonization with an infection rate of 75% (12/16). Similarly, 50% of *K. pneumoniae* isolates were MDR, while only 31% of isolates of *E. coli* were MDR (Temperoni *et al*., (2021).

Extended-spectrum beta-lactamases (ESBLs) are serine β-lactamases, characterized by their ability to hydrolyse expanded spectrum β-lactam antibiotics. They confer resistance to most β-lactam antibiotics, including expanded-spectrum cephalosporins and monobactams, but not to carbapenems and cephamycins. ESBLs are inhibited by β-lactamase inhibitors such as clavulanate (CA), sulbactam, or tazobactam (Bush and Jacoby, 2010). ESBL production has been found in in many genera of Enterobacterales especially *Escherichia coli*, *Klebsiella* spp. and *Proteus mirabilis*, *Enterobacter* spp., *Providencia stuartii* as well as *Pseudomonas aeruginosa, a* Pseudomonadale (Teklu *et al*., 2019; CLSI, 2020; Hosu *et al*., 2021). As a large group of plasmid-mediated, and rapidly evolving enzymes, ESBLs posing a major therapeutic challenge in the treatment of hospitalized and community-based patients.

In Nigeria, antibiotic use in poultry farming is very common (Adebowale *et al*., (2016). The use of antibiotics in animal production is one of the key factors leading to the emergence of resistant strains. Resistant bacteria have been isolated from chicken and cow wastes (Ogbor *et al*., 2019; Omojowo and Omojasola, 2013). When humans consume animal products contaminated with resistant pathogens, they are transferred to humans through the food chain. Studies have shown that waste from poultry harbor antibiotic resistance microbes. Ogbor *et al*., (2019) found that 5.5% of samples of chicken droppings harbored *Campylobacter coli* and all the isolates were resistant to nalidixic acid, chloramphenicol, cloxacillin, and streptomycin. Accumulated poultry waste is used as organic fertilizer for farm crops in Nigeria. Cow dung harboring resistant bacteria have been used to fertilize a fish pond in Nigeria (Omojowo and Omojasola, 2013). The contamination of the environment and farm produce with waste from livestock is a risk factor for the spread of antimicrobial resistance to humans. The environment becomes a large source of antibiotic resistance genes (ARGs) for pathogenic bacteria, leading to the risk of infection due to multidrug resistant bacteria (Baron *et al*., 2018).

Most research on antimicrobial drug resistance has been focused on resistance in clinically relevant pathogenic bacteria (Hailemariam *et al*., 2021; Ennab *et al*., 2022; Adekunle *et al*.,

2021). However, a vast and largely unexplored reservoir of resistance genes is present in nonpathogenic bacteria living in the environment or as commensal agents (Heydari *et al*., 2022; Singh *et al*., 2018). The existence of resistant strains among normal microbiota has serious public health implications.

The present study aims to determine the antibiotic resistance patterns and ESBLs production of Gram-negative bacteria isolated from healthy individuals and livestock waste with a view to suggesting ways of mitigating the emergence and threat of resistant normal microbiota.

MATERIALS AND METHODS

Sample collection, Culture and Isolation

Twenty-three (23) samples were collected in February, 2022 and included skin swabs (6), nasal swabs (4), urine (6), and stool (3) from healthy adult humans; chicken droppings (2) from OAUSTECH poultry; and cattle droppings (2) from the abattoir at Okitipupa Main Market. The samples were collected aseptically and analysed according to microbiological standards (CLSI, 2020).

Bacteria was isolated from the samples by growing on selective and differential bacteriological media including MacConkey agar, Eosin methylene blue agar (EMB), and Xylose lysine deoxycholate agar (XLD) and Chocolate agar, at 37^{o}C for 18-24 h. Suspected colonies were subjected to Gram-staining and Gram-negative colonies were sub-cultured onto Nutrient agar and incubated at 37^{o}C for 18-24 h to purify the isolates and subsequently stored at 4^{o}C

Identification and Antimicrobial Susceptibility Tests (AST) of bacterial Isolates

A total of nineteen (19) isolates of Gram-negative bacteria were isolated and submitted for VITEK® 2 analysis at Microbiology Laboratory, Lagos University Teaching Hospital (LUTH), Nigeria. For identification of the isolates, a stock culture of each Gram-negative bacterial isolate was sub-cultured on MacConkey agar plate and incubated at 37^{o}C for 18 hours. A loopful of the bacterium was transferred aseptically into 5 mL of sterile normal saline in a test tube to form a suspension. The bacterial suspension was standardized to 0.5 MacFarland turbidity standard using a turbidimeter.

Nineteen cards were attached to 19 test tubes containing the suspension of isolates to be identified, with the tip of the suction capillary tube of the card deeply immersed in the suspension. Each test tube was fixed to the cassette. The cassette was placed in the vacuum chamber of the system. Each VITEK 2 identification card has 64 wells (8 rows of 8 wells) which contain different dehydrated media required for different biochemical tests targeted at identifying different Gram-negative bacterial species or strains. A high vacuum was created inside the vacuum chamber, which forced the bacteria suspension to be sucked into the capillary tubes and dispensed into the wells of the cards. The cassette was taken out of the vacuum chamber and placed inside the incubation and analysis chamber and allowed to incubate at the prescribed temperature for a prescribed period of time as programmed by the control panel on the VITEK 2 compact system. The colour changes in all the wells were recorded automatically in the VITEK 2 compact system. The results of the colour changes went to a

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computer system attached to the VITEK 2 compact system which automatically compares the results with those available in its library for different bacteria and/or fungi, and finally gave the name of the bacteria with a definite probability (VITEK 2 Identification Card and Operation, September 4, 2021).

Antimicrobial Susceptibility Tests (AST)

Antimicrobial susceptibilities of 19 isolates were determined using the VITEK® 2 AST-GN75 Card, a Gram-negative susceptibility card (bioMerieux, Marcy L'Etoile, France). Each card contains multiple wells with increasing concentrations of various antibiotics in the broth, and at least one positive control well with growth-promoting broth and no antibiotic. The bacterial suspension was coupled with ATS card and was automatically filled, sealed and placed into VITEK 2 instrument. Growth in the positive control well was monitored until a pre-determined minimum amount of bacterial growth was detected through turbidity measurements (i.e. percent change of raw transmittance units – %ΔRTU). Growth in the control well showed that the test isolate is viable and growing at an appropriate rate. This analysis continued every 15 minutes until the susceptibility test was completed. The MIC was determined by comparing the growth of the isolate to the growth of isolates with known MICs (Badger-Emeka *et al*., 2018; Sanders *et al.*, 2000; Sanders *et al*., 2001; Michalik, 2017).

Calculation of Multiple antibiotic resistance (MAR) Index

The multiple antibiotics resistance (MAR) index was determined for each of the selected bacterial isolates using a formula MAR = a/b, where a is the number of antibiotics to which the test isolate displayed resistance and b is the total number of antibiotics against which the test organism has been evaluated for sensitivity (Afunwa *et al*., 2020).

Expanded-spectrum β-lactamase (ESBL) testing

The Gram-negative isolates (n=19) were tested on the VITEK-2 ESBL Card (bioMérieux). The card contained a ESBL test panel having six wells containing cefepime at 1.0 µg/ml, cefotaxime at 0.5 µg/ml, and ceftazidime at 0.5 µg/ml, alone and in combination with clavulanate (CA) (10, 4, and 4 µg/ml, respectively). The wells were automatically inoculated with test organisms. The growth in each well was quantitatively assessed by means of an optical scanner. The proportional reduction in growth in wells containing cephalosporin plus CA compared with those containing the cephalosporin alone is considered indicative of ESBL production (Spanu *et al*., 2006).

RESULTS

Properties of VITEK 2 Analysis of bacterial isolates

The properties of the VITEK® 2 and AES analyses of Gram-negative isolates are presented on Table 1.

The probability that the isolates were correctly identified ranged from 91% to 99% with *Escherichia coli* and *Acinetobacter baumannii* having the highest probabilities. The AES findings showed that minimal inhibitory concentrations were consistent for 16 isolates, which means that the isolates are compatible with their phenotypic resistant pattern. The exceptions were *A. baumannii* 13, *A. haemolyticus* 27 and *Providentia stuartii* 28 which showed inconsistent result or analysis not performed respectively.

Table 1: VITEK-2 Analysis of bacterial isolates

Name of Isolate/LAB ID	Analysis Time (hours)	Probability	Status	AES Findings (Confidence)
E. coli 12	6.80	91%	Final	Consistent
E. coli 14	3.85	99%	Final	Consistent
E. coli 19	5.77	97%	Final	Consistent
E. coli 21	4.77	95%	Final	Consistent
E. coli 23	4.02	99%	Final	Consistent
E. coli 31	3.88	99%	Final	Consistent
E. coli 32	3.83	99%	Final	Consistent
E. coli 33	3.93	99%	Final	Consistent
E. coli 34	4.83	91%	Final	Consistent
Klebsiella pneumoniae ssp pneumoniae 015	4.82	91%	Final	Consistent
Enterobacter cloacae ssp dissolvens 15A	4.80	91%	Final	Consistent
Acinetobacter baumannii 6	5.87	99%	Final	Consistent
A. baumannii 11	4.87	99%	Final	Consistent
A. baumannii 13	4.85	-	Final	Inconsistent
A. haemolyticus 27	4.87	-	Final	Analysis not performed
Providentia stuartii 25	5.95	92%	Final	Consistent
Providentia stuartii 26	5.82	94%	Final	Consistent
Providentia stuartii 28	5.85	92%	Final	Inconsistent
Providentia stuartii 30	4.90	97%	Final	Consistent

Results of Antimicrobial Susceptibility Tests (AST) by VITEK® 2 and Advanced Expert System (AES)

The results of antimicrobial screening of bacterial isolates are presented on Table 2.

Nineteen (19) Gram-negative bacterial isolates were screened against 17 antibiotics by determining their minimal inhibitory concentrations (MIC) in µg/ml. The MICs were interpreted as susceptible (S) intermediately susceptible (I) or resistant (R). Overall, the isolates showed the highest susceptibilities to Ertapenem, Amikacin, Ciprofloxacin and Levofloxacin (100%); Ceftazidime, Cefepime, Meropenem (94.7%); Cefoxitin (93.3%); Gentamicin and Tobramycin (73.7%). These were followed by Trimethoprim /Sulfamethoxazole (68.4%); Nitrofurantoin (66.7%); Cefazolin (52.6%); Ampicillin/Sulbactam (38.9%); Piperacillin (36.8%) and Ampicillin (21.4%). Some isolates showed intermediate susceptibility to Ampicillin/Sulbactam (22.2%); Nitrofurantoin (6.7%); and Ceftriaxone (5.3%). Resistances to the antibiotics were in the order of Ampicillin (78.6%); Piperacillin (63.2%); Cefazolin (47.4%); Ampicillin/Sulbactam (38.9%); Trimethoprim/Sulfamethoxazole (31.6%); Nitrofurantoin (26.7%); Gentamicin and Tobramycin (26.3%); Ceftriaxone (15.8%); Cefoxitin (6.7%); Ceftazidime, Cefepime, and Meropenem (5.3%).

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Table 2: Minimal Inhibitory Concentrations (µg/mL) of antibiotics against Bacterial Isolates

Isolate	ESBL	Ampicillin	Ampicillin /Sulbactam	Piperacillin	Cefazolin	Cefoxitin	Ceftazidime	Ceftriaxone	Cefepime	Ertapenem	Meropenem	Amikacin	Gentamicin	Tobramycin	Ciprofloxacin	Levofloxacin	Nitrofurantoin	Trimethoprim/ Sulfamethoxazole	MDR
E. coli 12	NEG	≥ 32 R	= 8 S	≥ 128 R	≤ 4 S	≤ 4 S	≤ 1 S	≤ 1 S	≤ 1 S	≤ 0.5 S	≤ 0.25 S	≤ 2 S	≤ 1 S	≤ 1 S	≤ 0.25 S	= 1 S	≤ 16 S	≤ 20 S	-
E. coli 14	NEG	≥ 32 R	≥ 32 R	≥ 128 R	≤ 4 S	≤ 4 S	≤ 1 S	≤ 1 S	≤ 1 S	≤ 0.5 S	≤ 0.25 S	≤ 2 S	≤ 1 S	≤ 1 S	= 1 S	= 1 S	≤ 16 S	≥ 320 R	-
E. coli 19	NEG	= 8 S	= 4 S	≤ 4 S	≤ 4 S	≤ 4 S	≤ 1 S	≤ 1 S	≤ 0.5 S	≤ 0.5 S	≤ 0.25 S	≤ 2 S	≤ 1 S	≤ 1 S	≤ 0.25 S	≤ 0.12 S	≤ 16 S	≤ 20 S	-
E. coli 21	NEG	≥ 32 R	= 16 I	≥ 128 R	≤ 4 S	≤ 4 S	≤ 1 S	≤ 1 S	≤ 0.5 S	≤ 0.5 S	≤ 0.25 S	≤ 2 S	≥ 16 R	= 8 *R	≤ 0.25 S	= 1 S	≤ 16 S	≥ 320 R	+
E. coli 23	NEG	= 4 S	= 4 S	≤ 4 S	≤ 4 S	≤ 4 S	≤ 1 S	≤ 1 S	≤ 0.5 S	≤ 0.5 S	≤ 0.25 S	≤ 2 S	≤ 1 S	≤ 1 S	≤ 0.25 S	= 1 S	≤ 16 S	≤ 20 S	-
E. coli 31	NEG	≥ 32 R	= 16 I	≥ 128 R	≤ 4 S	≤ 4 S	≤ 1 S	≤ 1 S	≤ 0.5 S	≤ 0.5 S	≤ 0.25 S	≤ 2 S	≤ 1 S	≤ 1 S	≤ 0.25 S	≤ 0.12 S	≤ 16 S	≥ 320 R	-
E. coli 32	NEG	≤ 2 S	≤ 2 S	≤ 4 S	≤ 4 S	≤ 4 S	≤ 1 S	≤ 1 S	≤ 0.5 S	≤ 0.5 S	≤ 0.25 S	≤ 2 S	≤ 1 S	≤ 1 S	≤ 0.25 S	≤ 0.12 S	≤ 16 S	≤ 20 S	-
E. coli 33	NEG	≥ 32 R	= 16 I	≥ 128 R	≤ 4 S	≤ 4 S	≤ 1 S	≤ 1 S	≤ 0.5 S	≤ 0.5 S	≤ 0.25 S	≤ 2 S	≤ 1 S	≤ 1 S	≤ 0.25 S	≤ 0.12 S	≤ 16 S	≤ 20 S	-
E. coli 34	NEG	≥ 32 R	= 16 I	≥ 128 R	≤ 4 S	≤ 4 S	≤ 1 S	≤ 1 S	≤ 0.5 S	≤ 0.5 S	≤ 0.25 S	≤ 2 S	≥ 16 R	≥ 16 R	≤ 0.25 S	= 1 S	≤ 16 S	≥ 320 R	+
Klebsiella pneumoniae ssp pneumoniae 015	NEG	≥ 32 R	= 8 S	= 16 *R	≤ 4 S	≤ 4 S	≤ 1 S	≤ 1 S	≤ 1 S	≤0.5 S	≤ 0.25 S	≤ 2 S	≤ 1 S	≤ 1 S	≤ 0.25 S	≤ 0.12 S	= 32 S	= 40 S	-
Enterobacter cloacae ssp dissolvens 15A	-	-	-	= 8 S	≥ 64 R	≥ 64 R	≤ 1 S	≤ 1 S	≤ 1 S	≤ 0.5 S	≤ 0.25 S	≤ 2 S	≤ 1 S	≤ 1 S	≤ 0.25 S	≤ 0.12 S	= 64 I	≥ 320 R	**+
Acinetobacter baumannii 6	-	-	≤ 2 S	= 8 S	≥ 64 R	-	= 4 S	= 16 *R	= 2 S	-	≤ 0.25 S	-	≤ 1 S	≤ 1 S	≤ 0.25 S	≤ 0.12 S	-	≤ 20 S	-
A. baumannii 11	-	-	= 4 S	= 64 *R	≥ 64 R	-	= 8 S	= 32 *R	= 4 S	-	= 1 S	-	≤ 1 S	≤ 1 S	= 0.5 S	= 0.25 S	-	≤ 20 S	-
A. baumannii 13	-	-	≥ 32 R	≥ 128 R	≥ 64 R	-	= 32 R	≥ 64 R	= 4 S	-	= 1 S	-	= 2 S	= 2 S	= 0.5 S	= 2 S	-	≤ 20 S	-
A. haemolyticus 27	-	-	≥ 32 R	≥ 128 R	≥ 64 R	-	≤ 1 S	≤ 1 S	≤ 1 S	-	= 1 S	≤ 2 S	≤ 1 S	≤ 1 S	≤0.25 S	= 1 S	-	≥ 320 R	-
Providentia stuartii 25	-	= 16 *R	= 16 *R	≤ 4 S	≥ 64 R	≤ 4 S	≤ 1 S	≤ 1 S	≤ 1 S	≤ 0.5 S	≤ 0.25 S	≤ 2 S	≤ 1 S	= 2 *R	≤ 0.25 S	≤ 0.12 S	= 128 R	≤ 20 S	+
Providentia stuartii 26	-	≥ 32 R	≥ 32 R	≤ 4 *R	≥ 64 R	= 8 S	≤ 1 S	≤ 1 S	≤ 1 S	= 1* S	= 1 S	≤ 2 S	= 2 *R	= 2 *R	≤ 0.25 S	= 0.25 S	= 128 R	≤ 20 S	+
Providentia stuartii 28	-	≥ 32 R	≥ 32 R	≥ 128 R	≥ 64 R	= 8 S	≤ 1 S	= 2 I	≥ 64 R	= 1 S	= 4 R	= 4 S	= 4 S	= 4 S	≤ 0.25 S	= 0.25 S	= 128 R	= 40 S	+
Providentia stuartii 30	-	≥ 32 R	≥ 32 R	≤ 4 S	≥ 64 R	≤ 4 S	≤ 1 S	≤ 1 S	≤ 1 S	≤ 0.5 S	= 0.5 S	≤ 2 S	= 4 S	= 4 *R	≤ 0.25 S	≤ 0.12 S	= 128 R	≤ 20 S	+
% S		21.4	38.9	36.8	52.6	93.3	94.7	78.9	94.7	100	94.7	100	73.7	73.7	100	100	66.7	68.4	
% I		0	22.2	00	0	0	0	5.3	0	0	0	0	0	0	0	0	6.7	0	
% R		78.6	38.9	63.2	47.4	6.7	5.3	15.8	5.3	0	5.3	00	26.3	26.3	0	0	26.7	31.6	

S- susceptible, I- intermediately susceptible, R- resistant, MDR- multiple drug resistance, ** -verge of MDR, * AES modified, - Antibiotics not tested (VITEK 2 and AES has the ability select only the antibiotics required for each species).

Multiple drug resistance (MDR) of isolates

The data on Table 2 shows that out of 19 bacterial isolates, only 7 isolates exhibited multiple drug resistance (resistance to three or more classes of antibiotics). These included two *Escherichia coli* isolates (*E. coli* 21 and *E. coli* 34) which showed MDR to β-lactams, aminoglycosides and Trimethoprim/Sulfamethoxazole; all four *Providentia stuartii* isolates exhibited MDR to three or more classes of antibiotics; *Enterobacter cloacae* ssp *dissolvens* was found to be on the verge of becoming MDR.

Susceptibility Profiles of *Klebsiella pneumoniae* ssp. *pneumoniae* and *Enterobacter cloacae* ssp. *dissolvens*

Antimicrobial susceptibilities of *Klebsiella pneumoniae* ssp *pneumoniae* and *Enterobacter cloacae* ssp *dissolvens* are presented on Table 2.

Klebsiella pneumoniae ssp *pneumoniae* (n = 1) was susceptible to 88.2% (15/17) of the antibiotics including Ampicillin/Sulbactam, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, and Trimethoprim/ Sulfamethoxazole. *Klebsiella pneumoniae* ssp *pneumoniae* was resistant to 11.8% (2/17) of the antibiotics including Ampicillin and Piperacillin.

Enterobacter cloacae ssp *dissolvens* (n = 1) was susceptible to 64.7% (11/17) of the antibiotics including Piperacillin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, while intermediately susceptible to Nitrofurantoin. *Enterobacter cloacae* ssp *dissolvens* was resistant to 17.6% (3/17) of the antibiotics including Cefazolin, Cefoxitin, and Trimethoprim/ Sulfamethoxazole.

Susceptibilities of *Escherichia coli*, *Acinetobacter baumannii and Providentia stuartii*

Antimicrobial susceptibilities of *Escherichia coli*, *Acinetobacter baumannii and Providentia stuartii* are presented on Table 3.

Escherichia coli isolates (n=9) were susceptible to eleven antibiotics including all the tested cephalosporins, carbapenems, Fluoroquinolones, Amikacin, and Nitrofurantoin (100%); Gentamicin and Tobramycin (77.8%); Trimethoprim/ Sulfamethoxazole (55.6%); Ampicillin/ Sulbactam (44.4%); Ampicillin and Piperacillin (33.3%). *E. coli* isolates were resistant to Ampicillin and Piperacillin (66.7%); Trimethoprim/Sulfamethoxazole (44.4%); Gentamicin and Tobramycin (22.2%); Ampicillin/Sulbactam (11.1%).

Acinetobacter spp. (n=4) were susceptible to Cefepime, Meropenem, Gentamicin, Tobramycin, Ciprofloxacin, and Levofloxacin (100%); Ceftazidime, and Trimethoprim/Sulfamethoxazole (75%); Ampicillin/Sulbactam (50%); Piperacillin, Ceftriaxone, and Amikacin (25%). *Acinetobacter* spp. were resistant to Cefazolin (100%); Piperacillin and Ceftriaxone (75%); Ampicillin/Sulbactam (50%); Ceftazidime, and Trimethoprim/ Sulfamethoxazole (25%).

Providentia stuartii (n = 4) were susceptible to Cefoxitin, Ceftazidime, Ertapenem, Amikacin, Ciprofloxacin, Levofloxacin and Trimethoprim/Sulfamethoxazole (100%); Ceftriaxone, Cefepime, and Meropenem (75%), Piperacillin (50%); Gentamicin and Tobramycin (25%). *Providentia stuartii*

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isolates were resistant to Ampicillin, Ampicillin/Sulbactam, Cefazolin and Nitrofurantoin (100%); Gentamicin and Tobramycin (75%); Piperacillin (50%); Cefepime and Meropenem (25%).

Table 3: Percentage susceptibilities of *E. coli, A. baumannii and Providentia stuartii* to the antibiotics

Isolate	Ampicillin	Ampicillin /Sulbactam	Piperacillin	Cefazolin	Cefoxitin	Ceftazidime	Ceftriaxone	Cefepime	Ertapenem	Meropenem	Amikacin	Gentamicin	Tobramycin	Ciprofloxacin	Levofloxacin	Nitrofurantoin	Trimethoprim/ Sulfamethoxazole
***E. coli* (n = 9)**																	
%S	33.3	44.4	33.3	100	100	100	100	100	100	100	100	77.8	77.8	100	100	100	55.6
%I	0	44.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
%R	66.7	11.1	66.7	0	0	0	0	0	0	0	0	22.2	22.2	0	0	0	44.4
***Acinetobacter* species (n = 4)**																	
%S	-	50	25	0	-	75	25	100	-	100	25	100	100	100	100	-	75
%I	-	0	0	0	-	0	0	0	-	0	-	0	0	0	0	-	0
%R	-	50	75	100	-	25	75	0	-	0	-	0	0	0	0	-	25
***Providentia stuartii* (n = 4)**																	
%S	0	0	50	0	100	100	75	75	100	75	100	25	25	100	100	0	100
%I	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0	0
%R	100	100	50	100	0	0	0	25	0	25	0	75	75	0	0	100	0

Source and Multiple Antibiotic Resistance (MAR) Index of Isolates

Table 4: Source and MAR index values of isolates

Isolate	Source	MAR Index
E. coli 12	Cattle droppings	0.12
E. coli 14	Stool	0.24
E. coli 19	Stool	0.00
E. coli 21	Chicken droppings	0.29
E. coli 23	Stool	0.00
E. coli 31	Stool	0.18
E. coli 32	Stool	0.00
E. coli 33	Cattle droppings	0.12
E. coli 34	Chicken droppings	0.29
Klebsiella pneumoniae ssp pneumoniae 015	Stool	0.12
Enterobacter cloacae ssp dissolvens 15A	Stool	0.20
Acinetobacter baumannii 6	Urine	0.17
A. baumannii 11	Skin swab	0.25
A. baumannii 13	Stool	0.42
A. haemolyticus 27	Stool	0.31
Providentia stuartii 25	Skin swab	0.35
Providentia stuartii 26	Nasal swab	0.41
Providentia stuartii 28	Skin swab	0.41
Providentia stuartii 30	Nasal swab	0.35

The sources and MAR index values of the isolates are presented on Table 4.

The MAR index values of the isolates varied in an irregular pattern. 33.3% (3/9) of *E. coli* isolates (*E. coli* 19, *E. coli* 23, *E. coli* 32) had MAR index values of 0.00, indicating that they were susceptible to all the antibiotics tested. 33.3% (3/9) of *E. coli* isolates, 75% (3/4) of *Acinetobacter* isolates and 100% (4/4) of *Providentia stuartii* isolates had MAR index values above the critical limit of 0.2. Overall, 52.6% (10/19) of all the isolates had MAR index values above 0.2 (ranging from 0.24 to 0.42). The highest MAR index values above 0.2 were shown by *Acinetobacter baumannii* (0.25 – 0.42) and *Providentia stuartii* (0.35 – 0.41) isolated from stool, skin swab and nasal swab. These were followed by *E. coli* 21 and *E. coli* 34 isolates from chicken droppings (MAR index = 0.29).

Results of ESBL Production Test

VITEK 2 system tested *E. coli* isolates and *Klebsiella pneumoniae* ssp *pneumoniae* for ESBL production. The result showed that all of these isolates tested negative for ESBL production. The other isolates were not tested for their ESBL production.

DISCUSSION

The identification and antimicrobial susceptibility testing of Gram-negative isolates by VITEK 2 in this study is in consonance with other authors ((Badger-Emeka *et al*., 2018; Sanders *et al*., 2001; Michalik, 2017; Spanu *et al*., 2006). Nineteen (19) Gram-negative isolates were identified. Sixteen (16) isolates were identified with a probability range of 91% to 99% while *A. baumannii* 13, *A. haemolyticus* 27 and *Providentia stuartii* 28 showed inconsistent result or analysis not performed respectively. Gram-negative bacteria are responsible for several infections and are becoming increasingly **multiple drug resistant (MDR)**, limiting therapeutic options in infection management. Rapid and accurate identification of MDR strains by VITEK® 2 automated system is crucial for the success of antimicrobial therapy and preventing the spread of these organisms.

The high percentage of susceptibilities exhibited in this study to Ertapenem, Amikacin, Ciprofloxacin and Levofloxacin, Ceftazidime, Cefepime, Meropenem, Cefoxitin, Gentamicin and Tobramycin shows that these antibiotics can still serve as effective agents in the therapy of Gram-negative infections. Significant resistance to Ampicillin and Piperacillin, and low to moderate resistance to other antibiotics, reveals both advanced and emerging resistance strains.

E. coli isolates showed reduced resistance in this study to Ampicillin and Piperacillin, Trimethoprim/Sulfamethoxazole, Gentamicin and Tobramycin, and Ampicillin/Sulbactam. This is in agreement with the findings of a hospital-based study in which Hailemariam *et al*. (2021) reported a high resistance of *E. coli* to ciprofloxacin (63.6%) and cotrimoxazole (70.4%). These findings suggest that resident *E. coli* in healthy individuals may be more susceptible to antibiotics than isolates from hospital samples. This is supported by the findings of other authors (Galarde-López *et al*., 2022). *Acinetobacter* spp. showed high resistance to Cefazolin (100%); and Piperacillin and Ceftriaxone (75%). These results are similar to previous reports (Badger-Emeka *et al*., 2018). Similarly, Al-Tamimi *et al*., (2022) reported high resistances of *A. baumannii* to cephalosporins and fluoroquinolones.

Providentia stuartii isolates showed the highest level of resistance in the study. All the four isolates were 100% resistant to 4 antibiotics - Ampicillin, Ampicillin/

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Sulbactam, Cefazolin and Nitrofurantoin; They also showed resistance to Gentamicin and Tobramycin (75%). This is similar to report of Liu *et al*., (2020). *Providencia* species are Gram-negative bacilli in the Enterobacteriaceae family. The bacterium is a known drug resistance opportunistic pathogen that causes healthcare-associated infections, such as acute enteric infection, urinary tract infection, and lung diseases. The organism is typically isolated from human secretions, including urine, sputum, throat swab, blood, stool, and wound secretion, and pus. The treatment of choice is based on antibiotic sensitivities, infection source, and comorbid conditions (Abdallah *et al*., 2018; Woreta *et al*., 2022; Liu *et al*., 2020; Lin *et al*., 2017). *Klebsiella pneumoniae* ssp *pneumoniae* in the present study was found to be resistant to Ampicillin and Piperacillin, contrary to the findings of Hailemariam *et al*, (2021) who reported from Ethiopia high resistance levels of *K. pneumoniae* isolated from clinical samples to ceftazidime (82%) and ciprofloxacin (80.9), as well as ampicillin (75%). This suggests that bacteria isolated from clinical samples are more resistant to antibiotics than their counterparts among human resident. *Enterobacter cloacae* ssp *dissolvens* was resistant to Cefazolin, Cefoxitin, and Trimethoprim/ Sulfamethoxazole. Broad-spectrum antibiotic resistance, including the recent emergence of resistance to last-resort carbapenems, has led to increased interest in this group of organisms and carbapenem-resistant *E. cloacae* complex (CREC) in particular (Annavajhala *et al*., 2019). However, *Enterobacter cloacae* ssp. *dissolvens* isolated from resident bacteria in stool, was susceptible to Ertapenem and Meropenem.

MDR was defined as resistance of an isolate to three or more classes of antibiotics (Magiorakos *et al*., 2012). All four *Providentia stuartii* isolates exhibited MDR to three or more classes of antibiotics. Similarly, two *Escherichia coli* isolates (*E. coli* 21 and *E. coli* 34) showed MDR to β-lactams, aminoglycosides and Trimethoprim/Sulfamethoxazole. However, majority of the isolates did not exhibit MDR to the antibiotics. These include seven *E. coli* isolates, *Klebsiella pneumoniae*, and *Acinetobacter* spp.

The isolation and resistance of *E. coli*, *K. pneumoniae* and *A. baumannii* are of public health importance. These microbes are among six ESKAPE organisms identified as the leading cause of healthcare-acquired infections worldwide. Most of them are multidrug resistant isolates, which is one of the greatest challenges in clinical practice (Zeng *et al*., 2019; De Oliveira *et al*., 2020). This assertion is confirmed by findings in this study.

MAR index values above 0.2 were shown by *Acinetobacter baumannii* and *Providentia stuartii* and *E. coli* isolated from chicken droppings. MAR index values above the critical limit of 0.2 suggest that these bacteria originate from a high-risk source of contamination where several antibiotics are often used such as (Afunwa *et al*., 2020; Sandhu *et al*., 2016; Osundiya *et al*., 2013). High MAR index values among resident microbiota is an indication of high selective pressure and uncontrolled use of antibiotics.

The VITEK -2 system tested *E. coli* isolates and *Klebsiella pneumoniae* ssp. *pneumoniae* for ESBL production. All of these isolates were negative for ESBL production. ESBLs confer resistance to most β-lactam antibiotics, including expanded-spectrum cephalosporins

and monobactams (Bush and Jacoby, 2010). The ESLB-negative status of the isolates in the present study may explain the high levels of susceptibility shown by a significant number of the isolates, which seem to be more susceptible to antibiotics when compared with clinical isolates from hospital settings as reported by multiple authors (Temperoni *et al*., 2021; Zeng *et al*., 2019; De Oliveira *et al*., 2020).

CONCLUSION

Gram-negative bacteria resident in healthy individuals harbour antibiotic resistance which have public health importance, having potential of causing community-based, resistant opportunistic infections difficult to treat. Resident bacteria in healthy individuals may acquire resistant traits during horizontal gene transfer in the environment, or due to selective pressure during antibiotic therapy or in the course of drug-abuse through self-prescription. Proper disposal of body secretions from healthy individuals is a necessity. Inappropriate antibiotic prescription in the hospital setting, community-based drug abuse and uncontrolled use of antibiotics in livestock production all promote the emergence of resistant strains including normal microbiota residing in healthy individuals. Appropriate restrictions to antibiotic usage and pretreatment of livestock waste before use as manure are recommended in the fight against resistant bacteria.

Ethical Approval

Ethical approval was not required and the samples were voluntarily donated by healthy participants.

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REFERENCES

Abdallah, M., Alhababi, R., Alqudah, N., Aldyyat, B. and Alharthy, A. (2018) First report of carbapenem-resistant *Providencia stuartii* in Saudi Arabia. *New Microbes and New Infections 26: 107–109*. https://doi.org/10.1016/j.nmni.2018.09.007

Adebowale, O.O., Adeyemo, O.K., Awoyomi, O., Dada, R. and Adebowale, O. (2016) Antibiotic use and practices in commercial poultry laying hens in Ogun State, Nigeria. *Revue Délevage et de Medecine Veterinaire Des Pays Tropicaux 69(1): 41-45*.

Adekunle, O.C., Mustapha, A., Odewale, G. and Ojedele, R.O. (2022) Detection of antibiotic resistance genes among multiple drug resistant *Pseudomonas aeruginosa* strains isolated from clinical sources in selected health institutions in Kwara State. *Research Journal of Health Sciences 10(1): January/March 2022*. DOI: 10.4314/rejhs.v10i1.5

Afunwa, R., Ezeanyinka, J., Afunwa, E., Udeh, A., Oli, A. and Unachukwu, M. (2020) Multiple Antibiotic Resistant Index of Gram-Negative Bacteria from Bird Droppings in Two Commercial Poultries in Enugu, Nigeria. *Open Journal of Medical Microbiology 10:171-181*. DOI: 10.4236/ojmm.2020. 104015.

Al-Tamimi, M., Albalawi, H., Alkhawaldeh, M., Alazzam, A., Ramadan, H., Altalalwah, M., Alma'aitah, A., Al Balawi, D., Shalabi, S., Abu-Raideh, J., et al. (2022) Multidrug-Resistant *Acinetobacter baumannii* in Jordan. *Microorganisms 10:849*. https://doi.org/10.3390/microorganisms10050849

Annavajhala, M.K., Gomez-Simmonds, A. and Uhlemann, A.C. (2019) Multidrug-Resistant Enterobacter cloacae Complex

Scientia Africana, Vol. 21 (No. 2), August, 2022. Pp 75-90 https://dx.doi.org/10.4314/sa.v21i2.8
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Emerging as a Global, Diversifying Threat. *Frontiers in Microbiology 10, 2019*. ISSN=1664-302X. https://www.frontiersin.org/article/10.3389/fmicb.2019.00044. DOI=10.3389/fmicb.2019.00044

Badger-Emeka, L-B., Alsultan, A.A., Al Rashed, A.S., Albarjas, A. and Alhaddad, M. (2018) Antimicrobial susceptibility pattern of Gram-negative bacteria isolated from intensive care units in Al-Ahsa, Kingdom of Saudi Arabia. *African Journal of Microbiology Research 12(31): 747-753*. DOI: 10.5897/AJMR 2018.8925

Baron, S.A., Diene, S.M., and Rolain, J.M. (2018) Human microbiomes and antibiotic resistance. *Human Microbiome Journal 10:43-52*. ISSN 2452-2317, https://doi.org/10.1016/j.humic.2018.08.005.

Baumgartner, M., Bayer, F., Pfrunder-Cardozo, K. R., Buckling, A. and Hall, A.R. (2020) Resident microbial communities inhibit growth and antibiotic-resistance evolution of *Escherichia coli* in human gut microbiome samples *PLoS Biology 18(4): e3000465*. DOI:10.1371/journal. pbio. 3000465.

Bush, K. and Jacoby, G.A. (2010) Updated functional classification of β-lactamases. *Antimicrobial Agents and Chemotherapy, 54:969-76*.

Cheng, G., Hu, Y., Yin, Y., Yang, X., Xiang, C., Wang, B. *et al.* (2012) **Functional screening of antibiotic resistance genes from human gut microbiota reveals a novel gene fusion.** *FEMS Microbiology Letters 336: 11-16*. DOI:10.1111/j.1574-6968.2012.02647.x

Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th Ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020:M100–Ed 30.

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility testing. In: *CLSI Supplement M100*. 31st ed. Vol. 41. No.3 Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2021: M100–Ed 31.

Coates, M., Lee, M. J., Norton, D. and MacLeod, A.S. (2019) The Skin and Intestinal Microbiota and Their Specific Innate Immune Systems. *Frontiers in Immunology 10:2019*. DOI=10.3389/ fimmu.2019.02950. ISSN=1664-3224.

Colavecchio, A., Cadieux, B., Lo, A. and Goodridge, L.D. (2017) Bacteriophages Contribute to the Spread of Antibiotic Resistance Genes among Foodborne Pathogens of the Enterobacteriaceae Family – A Review, *Frontiers in Microbiology 8:2017*, DOI:10.3389/ fmicb.2017.01108. ISSN=1664-302X

De Oliveira, D.M.P., Forde, B.M., Kidd, T.J., Harris, P.N.A., Schembri, M.A., Beatson, S.A., Paterson, D.L. and Walker, M.J. (2020) Antimicrobial resistance in ESKAPE pathogens. *Clinical Microbiology Reviews 33(3), e00181-e219*.

Ennab, R., Al-Momani, W., Al-Titi, R., and Elayan, A. (2022) Antibiotic Profile of Pathogenic Bacteria Isolated from Postsurgical Site Infections in Public Hospitals in Northern Jordan. *Infection and Drug Resistance 15:359–366*. http://doi.org/10.2147/IDR.S350406

Fancello, L., Desnues, C., Raoult, D., Rolain, J.M. (2011) **Bacteriophages and diffusion of genes encoding antimicrobial resistance in cystic fibrosis sputum microbiota.**

Journal of Antimicrobial Chemotherapy 66: 2448-2454. DOI: 10.1093/jac/dkr315

Galarde-López, M., Velazquez-Meza, M.E., Bobadilla-del-Valle, M., Cornejo-Juárez, P., Carrillo-Quiroz, B.A., Ponce-de-León, A., Sassoé-González, A., Saturno-Hernández, P. and Alpuche-Aranda, C.M. (2022) Antimicrobial Resistance Patterns and Clonal Distribution of E. coli, Enterobacter spp. and Acinetobacter spp. Strains Isolated from Two Hospital Wastewater Plants. *Antibiotics 11: 601*. https://doi.org/10.3390/ antibiotics11050601

Gashaw, M., Marame, Z.H., Abera, M. and Ali, S. (2021) Assessment of Gut Bacteria Profile and Antibiotic Resistance Pattern Among Psychotropic Drug Users: Comparative Cross-Sectional Study. *Infection and Drug Resistance 14:1875-1881*. https://doi.org/ 10.2147/IDR. S305992

Hailemariam, M., Alemayehu, T., Tadesse, B., Nigussie, N., Agegnehu, A., Habtemariam, T., Ali, M., Mitiku, E. and Azerefegne, E. (2021) Major bacterial isolate and antibiotic resistance from routine clinical samples in Southern Ethiopia. *Scientific Reports 11: 19710*. https://doi.org/10. 1038/s41598-021-99272-2

Heydari, A., Kim, N.D., Horswell, J., Gielen, G., Siggins, A., Taylor, M., Bromhead, C. and Palmer, B.R. (2022) Co-Selection of Heavy Metal and Antibiotic Resistance in Soil Bacteria from Agricultural Soils in New Zealand. *Sustainability 14: 1790*. https://doi.org/ 10.3390/su14031790

Hillman, E.T., Lu, H., Yao, T. and Nakatsu, C.H. (2017) Microbial Ecology along the Gastrointestinal Tract. *Microbes and environments 32(4), 300–313*. https://doi.org/10.1264/jsme2.ME17017

Hosu, M.C., Vasaikar, S.D., Okuthe, G.E. and Apalata, T. (2021) Detection of extended spectrum beta-lactamase genes in *Pseudomonas aeruginosa* isolated from patients in rural Eastern Cape Province, South Africa. *Scientific Reports 11: 7110*. DOI: 10.1038/s41598-021-86570-y

Jernberg, C., Lofmark, S., Edlund, C. and Jansson, J.K. (2010) Longterm impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology 156: 3216–3223*.

Kazimierczak, K.A. and Scott, K.P. (2007) Antibiotics and resistance genes: influencing the microbial ecosystem in the gut. *Advances in Applied Microbiology 62: 269–292*.

Li, Q., Chang, W., Zhang, H., Hu, D. and Wang, X. (2019) The Role of Plasmids in the Multiple Antibiotic Resistance Transfer in ESBLs-Producing *Escherichia coli* Isolated from Wastewater Treatment Plants. *Frontiers in Microbiology 10: 633*. DOI: 10.3389/fmicb.2019.00633

Lin, K., Lin, A.N., Linn, S., Reddy, M. and Bakshi, A. (2017) Recurrent Primary Suprahepatic Abscess Due to *Providencia Stuartii*: A Rare Phenomenon. *Cureus 9(9), e1691*. https://doi.org/10.7759/ cureus.1691

Liu, J., Wang, R., Fang, M. (2020) Clinical and drug resistance characteristics of *Providencia stuartii* infections in 76 patients. *Journal of International Medical Research 48(10):300060520962296*. doi: 10.1177/0300060520962296

Magiorakos, A.-P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F.,

Scientia Africana, Vol. 21 (No. 2), August, 2022. Pp 75-90 *https://dx.doi.org/10.4314/sa.v21i2.8*
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Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T. and Monnet, D.L. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection 18(3): 268-281*. ISSN 1198-743X, https://doi.org/10.1111/j.1469-0691.2011.03570.x.

Michalik, S. (2017) How does VITEK® 2 Generate MIC Values? https://www.biomerieux-microbio. com/how-does-vitek-2-generate-mic-values/

Ogbor, O., Ajayi, A., Zautner. A.E. and Smith, S.I. (2019) Antibiotic Susceptibility Profiles of *Campylobacter coli* Isolated from Poultry Farms in Lagos Nigeria – A Pilot Study. *European Journal of Microbiology & Immunology 9(2):32-34*. Doi: 10.1556/1886.2019. 00007

Omojola, F.S. and Omojasola, F.P. (2013) Antibiotic resistant pattern of bacterial pathogens isolated from cow dung used to fertilize Nigerian fish ponds. *Notulae Scientia Biologicae 5(1): 15-19*. DOI: 10.15835/nsb.5.1.8998

Osundiya, O.O., Oladele, R.O. and Oduyebo, O.O. (2013) Multiple Antibiotic Resistance (MAR) indices of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital. *African Journal of Clinical and Experimental Microbiology 14(3): 164-168*. doi: 10.4314/ajcem.v14i3.8

Partridge, S.R., Kwong, S.M., Firth, N., Jensen, S.O. (2018) Mobile Genetic Elements Associated with Antimicrobial Resistance. *Clinical Microbiology Reviews 31(4):e00088-17*. DOI:10.1128/CMR.00088-17

PLoS Human Microbiome Project Collection Manuscript Summaries Archived 4 March 2014 at the Wayback Machine,13 June 2012.

Rolain, J-M. (2013) **Food and human gut as reservoirs of transferable antibiotic resistance encoding genes**. *Frontiers in Microbiology 4: 73*. DOI:10.3389/fmicb.2013.00173

Sanders, C.C., Peyret, M., Moland, E.S., Shubert, C., Thomson, K.S., Boeufgras, J.M. and Sanders, W.E. Jr. (2000) Ability of the VITEK 2 advanced expert system to identify beta-lactam phenotypes in isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa*. *Journal of Clinical Microbiology 38(2):570-4*. doi: 10.1128/JCM.38.2.570-574.2000. PMID: 10655347; PMCID: PMC86150.

Sanders, C.C., Peyret, M., Moland, E.S., Cavalieri, S.J., Shubert, C., Thomson, K.S., Boeufgras, J.M. and Sanders, W.E. Jr. (2001) Potential impact of the VITEK 2 system and the Advanced Expert System on the clinical laboratory of a university-based hospital. *Journal of Clinical Microbiology 39(7):2379-85*. DOI: 10.1128/JCM.39.7.2379-2385. 2001. PMID: 11427542; PMCID: PMC88158.

Sandhu, R., Dahiya, S. and Sayal, P. (2016) Evaluation of Multiple Antibiotic Resistance (MAR) Index and Doxycycline Susceptibility of Acinetobacter Species among in patients. *Indian Journal of Microbial Research 3:299-304*.

Singh, A.K., Das, S., Singh, S., Gajamer, V.R., Pradhan, N., Lepcha, Y.D., and Tiwari, H.K. (2018) Prevalence of antibiotic resistance in commensal Escherichia coli among the children in rural hill communities of Northeast India. *PLoS*

One 13(6), e0199179. https://doi.org/10.1371/journal.pone.0199179

Spanu, T., Sanguinetti, M., Tumbarello, M., D'Inzeo, T., Fiori, B., Posteraro, B., Santangelo, R., Cauda, R. and Fadda, G. (2006) Evaluation of the new VITEK 2 extended-spectrum beta-lactamase (ESBL) test for rapid detection of ESBL production in Enterobacteriaceae isolates. *Journal of Clinical Microbiology 44(9), 3257–3262*. https://doi.org/10.1128/JCM.00433-06

Teklu, D.S., Negeri, A.A., Legese, M.H., Bedada, T.L., Woldemariam, H.K., and Tullu, K.D. (2019) Extended-spectrum beta-lactamase production and multi-drug resistance among *Enterobacteriaceae* isolated in Addis Ababa, Ethiopia. *Antimicrobial Resistance & Infection Control 8: 39*. https://doi.org/10.1186/s13756-019-0488-4

Temperoni, C., Caiazzo, L., Barchiesi, F. (2021) High Prevalence of Antibiotic Resistance among Opportunistic Pathogens Isolated from Patients with COVID-19 under Mechanical Ventilation: Results of a Single-Center Study. *Antibiotics 10(9):1080*. https://doi.org/10.3390/antibiotics10091080

Woreta, A.N., Kebede, H.B., Tilahun, Y., Teklegiorgis, S.G., and Abegaz, W.E. (2022) Antibiotic Susceptibility Pattern and Bacterial Spectrum Among Patients with External Eye Infections at Menelik II Referral Hospital in Addis Ababa, Ethiopia [Response to Letter]. *Infection and Drug Resistance 15:1967–1969*.

Zhen, X., Lundborg, C.S., Sun, X., Hu, X. and Dong, H. (2019) Economic burden of antibiotic resistance in ESKAPE organisms: A systematic review. *Antimicrobial Resistance & Infection Control 8(1): 137*.