

A Retrospective Study of the Antimicrobial Resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in Clinical Samples from a Healthcare Facility in Osogbo, Nigeria

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ABSTRACT

Background: *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are gram-negative bacteria that have been linked with various healthcare-associated and community-acquired infections. These three organisms have been identified as critical multi-drug resistant pathogens that require new drugs to combat their infections in humans. Due to the scarcity of data about the true burden of AMR in the 3 microorganisms in this locality, this study investigated the prevalence, pattern and trend of antimicrobial resistance exhibited by *K. pneumoniae*, *P. aeruginosa* and *E. coli* within Osogbo metropolis.

Method: Antimicrobial resistance prevalence, patterns and trends in *E. coli*, *K. pneumoniae* and *P. aeruginosa* isolates from clinical samples submitted for microbial culture and sensitivity test between January and December 2022 were analyzed. In this study, nine antibiotics including Cefixime, Cefotaxime, Cefuroxime, Ceftriaxone, Gentamicin, Augmentin, Imipenem, Ciprofloxacin and Levofloxacin were selected based on the spectrum of activity. Data analysis was done using SPSS version 25 for statistical analyses.

Results: There were 171, 83 and 32 isolates of *E. coli*, *K. pneumoniae* and *P. aeruginosa* identified in this study respectively. Overall, the three organisms showed higher AMR prevalence to IMP and ZEM, and least resistance to LBC. Essentially, 24 *P. aeruginosa*, 113 *E. coli*, and 59 *K. pneumoniae* isolates demonstrated multidrug resistance pattern. All *P. aeruginosa* isolates exhibited resistance to at least one antibiotic. AUG-CIP- CRO-CTX-CXM-GEN-IMP-LBC-ZEM resistance pattern occurred in both urine and wound samples for *P. aeruginosa*, as well as *E. coli* isolated from high vagina swab. Observed AMR in respect of carbapenem (IMP) and cephalosporin (cefuroxime, cefixime and cefotaxime) was above average for the 3 microorganisms. Patient age, nature and source of specimen are marginal factors influencing AMR of these isolates.

Conclusion: This study identified the high rate of resistance by the bacterial isolates most especially to cephalosporins. The high MAR-index suggests the practice of self-medication due to unrestricted access to over-the-counter medications. Hence, there is the need for effective campaigns to sensitize the public on the need to halt indiscriminate use of antibiotics, as well as advocate for laws and policies to guide the sale and purchase of antibiotics at the community level.

Keywords: Antimicrobial resistance, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, Microbial Culture Sensitivity, Prevalence, Clinical samples, Antibiotics

INTRODUCTION

Antimicrobial resistance (AMR) is integral part of one-health, with respect to the health of humans, animals, plants and the environment. Due to the existent bionetwork between humans, animals and the environment, AMR poses a global public health concern [1]. It has also been identified as a threat to the actualization of the sustainable developmental goals (SDGs) [2]. Recent report in many countries about antimicrobial resistance has highlighted an increase in antibiotics resistance, notably is the rate at which last-line antibiotics are rapidly failing [3]. There has been reported spike in cases of antimicrobial resistance among bacteria in the Family Enterobacteriaceae in recent time [4]. Carbapenem has always been restricted for the treatment of resistant bacteria, resistance to this class of antibiotics has been reported to be on the increase especially among Enterobacterales, Acinetobacter species, and *Pseudomonas aeruginosa* which are known to be responsible for serious bacterial health-care-associated infections [3].

Just like other public health concerns and epidemics, there are no actual or estimated threat of AMR in the country as there is dearth of data to determine the extent, trend and pattern of the situation in Nigeria [5]-[7]. In Nigeria, due to the poorly regulated antimicrobial market, antimicrobials are easily accessed and bought as over-the-counter medication without a prescription by a licensed and authorized medical practitioner [8]. Another factor is the almost non-existence of enforcement of prescription-only-access to antibiotics where required. The most worrisome in these scenarios is that access to over-the-counter drugs is not restricted to first or second-line antibiotics alone, but also applicable to the critically important class of antimicrobials [8]-[9]. Drivers of AMR include; human antimicrobial consumption, [10] environmental contamination [8], suboptimal diagnosis [11], agricultural use of antibiotics [12].

Escherichia coli, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are gram-negative bacteria [13]. Both *E. coli* and *K. pneumoniae* belong to the family Enterobacteriaceae [14]. *E. coli* have been linked with a variety of diseases, both at the community and hospital level [14]. *Klebsiella pneumoniae* is found in the environment [15], and has a history of rapid development of resistance to antibiotics [16]. *P. aeruginosa* is an encapsulated bacterium, it has a wide environmental presence and can cause infection in humans and animals, most especially in immunosuppressed individuals [17]-[18]. These 3 organisms made up 3 of the 4 organisms identified from Mapping Antimicrobial Resistance and Antimicrobial Use Partnership (MAAP) survey in 14 African countries, they are critical multi-drug resistant pathogens that requires new drugs to combat their infections in humans [19]. Globally, there has been a rapid increase in infections caused by multidrug-resistant (MDR) bacteria [20].

A systematic review to estimate the global burden of AMR published by the Lancet using the predictive statistical models shows that an estimated 4.95 million deaths were directly associated with bacterial resistance in 2019, this includes; 1.27 million deaths being attributed to AMR. In the same review, it was reported that at the regional level, western sub-Saharan Africa has highest all-age death rate of 27.3 deaths per 100 000 linked directly to resistance. Drug-pathogen resistance category of third-generation cephalosporin-resistant *K. pneumoniae*, multidrug-resistant third-generation cephalosporin-resistant *E. coli*, carbapenem resistant *K. pneumoniae*, fluoroquinolone-resistant *E. coli* caused 50 000–100 000 deaths each [7].

Prevalence of AMR and antibiogram of fluoroquinolone and carbapenem are understudied in Nigeria. Limited studies and data on antimicrobial resistance among the bacteria of interest are available in Osogbo. This has impeded the understanding of *P. aeruginosa*, *K. pneumoniae* and *E. coli* incidence and infection in human, all of which are imperative for the understanding of the burden of infection locally at the community level. This

has in a way restricted the public health professionals from implementing health education and awareness campaigns that can bring about reduction in the prevalence of AMR as well as promoting positive actions on appropriate use of antimicrobials. Hence, the focus of this study was to investigate antimicrobial resistance and drug resistance pattern exhibited by *K. pneumoniae*, *E. coli* and *P. aeruginosa*.

METHOD

The rates, pattern and trend of antimicrobial resistance in gram-negative bacteria, that were isolated between January and December, 2022 from clinical specimens including stool, urine, sputum, high vagina swab (HVS), semen, ear swab, blood, urethra swab, wound and endocervical swab. The collected specimens were processed in the microbiology unit of the medical laboratory of the healthcare facility. The Kirby-Bauer disk diffusion method was employed in performing antimicrobial sensitivity tests and antibiogram. Results were reported according to standard (Clinical Laboratory Standards Institute (CLSI) guidelines. The organisms included in this survey are *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Nine antibiotics were selected based on their spectrum of activity namely Cefixime (ZEM), Cefotaxime (CTX), Cefuroxime (CXM), Ceftriaxone (CRO), Gentamicin (GEN), Augmentin (AUG), Imipenem (IMP), Ciprofloxacin (CIP) and Levofloxacin (LBC). The sample size, gender and age group were predetermined and fixed, as only the patients with a positive culture of bacteria isolate of interest were included in the study. The data were extracted from the laboratory information system of the healthcare facility and included information on patients' age and gender, month of bacteria isolation, specimen type, antimicrobial susceptibility testing result.

Data analysis was done using Statistical Package for the Social Sciences version 25 software package (SPSS; IBM Corporation, Armonk, NY, USA) to analyze the extracted data. Findings were presented in table and graphs.

RESULTS

A total of 286 bacterial isolates were obtained in which infection rate of 53.8% and 46.2% were obtained from the male and female respectively. The age group included in this survey range from 0.1 through 100years, the highest frequency was found within 31-40 age group which represents 32.9% of total population size, the least frequency 0.3% was found in age group 91-100 (Table 1). Higher frequency of *K. pneumoniae* isolates (56.6%) was obtained from the female.

Isolates were cultured from various clinical specimens which include blood, semen, urine, HVS, wound etc. Urine and semen specimen formed the bulk of total specimen with the frequency of 24.1% each. Semen specimen was collected mainly from 3 age groups (21-30, 31-40 and 41-50) which makes up a total of 69.6% of total semen specimen, the same age group was responsible for majority of urine 68.1% and vagina swab 69.7% specimen. *E. coli* was isolated from all of the endocervical swab, stool and 87.5% of urethral swab specimens cultured in this study. Highest number of *K. pneumoniae* was isolated from sputum. Of the total wound specimen cultured in this study, *K. pneumoniae* was isolated from 61.5% while the highest number of *Pseudomonas aeruginosa*, 31.3% was also isolated from wound specimen. Only *K. pneumoniae* and *Pseudomonas aeruginosa* were isolated from blood and ear swab specimens in this present study. Bulk of the total *E. coli* isolates from this study were from semen (59), HVS (50) and urine (47) (Table 2).

From Table 3, Season was categorized into Wet and Dry season which was defined as; Wet season (April-October) and Dry season (November-March). A total number of 179 isolates were isolated during the wet season (*E. coli*, 111; *K. pneumoniae*, 50; and *P. aeruginosa*, 18) while 107 were isolated during the dry season (*E. coli*, 60; *K. pneumoniae*, 33; and *P. aeruginosa*, 14). All the 3 isolates had highest frequency in the wet

season.

Of the total 286 bacteria isolates isolated in this study, *Escherichia coli* accounted for 59.8% (171), *Klebsiella pneumoniae* 29.0% (83) and *Pseudomonas aeruginosa* 11.2% (32). *E. coli* has the highest frequency with *P. aeruginosa* having the least occurrence. There was no isolate of *Pseudomonas aeruginosa* that did not exhibit resistance to at least one of the antibiotics, 75% of *P. aeruginosa*, 66.1% of *E. coli* and 71.1% of *K. pneumoniae* demonstrated multidrug resistance pattern (Table 5).

It could be deduced from Table 4 that resistance was generally higher towards both IMP 55.6% and ZEM 54.9%. Susceptibility towards antibiotics was highest for GEN 59.8% and followed by CIP 54.9% and CTX 53.8%. Both *E. coli* and *P. aeruginosa* demonstrated highest resistance to ZEM at 58.5% and 71.9% respectively. Both also showed least resistance to LBC with *E. coli* having 53.1% and *P. aeruginosa* 25.0%. *Klebsiella pneumoniae* showed highest resistance towards IMP 57.8% and CXM 57.8% while it has least resistance to GEN 20.5% (Figure 2).

It was explicitly described in Figure 1, the antimicrobial resistance pattern in relation to the clinical specimens, 73.3% of isolates from blood specimen were resistant to CXM, and least resistance was to LBC 13.3%. Bacteria isolated from semen and urine demonstrated highest resistance to ZEM 60.9% and 57.9% respectively. Bacterial isolates from both semen and HVS also showed least resistance to LBC with bacteria from semen having 20.3%, urine 17.4% and HVS with the value of 16.7%. Of all the clinical samples in this study, isolates from wound has highest level of resistance to all the drugs; AUG 80.8%, ZEM, IMP and CXM 65.4% each, CTX 61.5% with the least resistance being to LBC 30.8%. Resistance to ZEM, IMP and CTX was at equal rate by bacterial isolates from ear swab at 85.7% each, least resistance to antibiotics by isolates from ear swab was towards LBC and GEN with 14.3%.

As shown in Table 6, the resistance pattern AUG-CIP-CRO-CTX-CXM-GEN-IMP-LBC-ZEM occurred in *P. aeruginosa* gotten from both urine and wound specimens, same pattern also has occurrence in *E. coli* isolated from HVS specimens. The pattern AUG-CTX-CXM-ZEM has occurrence in *E. coli* isolates cultured from both HVS and semen specimens, it occurred as well in *K. pneumoniae* isolated from wound specimen. AUG-CTX-CXM-IMP was found in *E. coli* isolated from semen and HVS specimens and also in *K. pneumoniae* from sputum specimen. AUG-CTX-CXM-IMP-ZEM resistance pattern was found to be common among *E. coli* isolates obtained from semen, urine, (UCS) and HVS specimen. For *K. pneumoniae* isolated from sputum and wound specimens, the pattern AUG-CIP-CRO-CTX-IMP-ZEM was found to be common.

In Table 7, ordinal regression model was performed. The parameter estimates in the table summarize the effect of each predictor of the dependent variable – bacteria isolates. Independent factors were the clinical specimens and multidrug (MDR) prevalence, while gender, age of the patients and seasons were the covariates. The signs of the coefficients for covariates and relative values of the coefficients for factor levels gave essential insights into the effects of the bacteria in the model. However, there was a significant association between the age of the patients and bacteria isolates. The positive coefficient as covariates implies direct relationships between predictors and outcomes ($\beta=0.271$, $df=1$, $p<0.05$). Other covariates such as gender ($\beta=0.341$, $df=1$, $p>0.05$) and the seasons ($\beta=-0.442$, $df=1$, $p>0.05$) were not statistically significant.

Considering the clinical specimen as a factor showing cumulative outcomes categories, ECS was insignificant with respect to bacteria isolates ($\beta=-0.403$, $df=1$, $p>0.05$). The clinical specimen – Semen ($\beta=16.726$, $df=1$, $p<0.05$) has a positive significant relationship with bacterial associates. There was a significant direct relationship between urine specimens and bacteria associates ($\beta=18.397$, $df=1$, $p<0.05$). Also, blood specimen had a positive and significant association with bacteria isolate ($\beta=22.123$, $df=1$, $p<0.05$). Sputum has a positive relationship with bacteria isolates, and the association was statistically significant ($\beta=19.814$, $df=1$, $p<0.05$).

There was also a significant relationship between HVS and bacteria isolates ($\beta=18.036$, $df=1$, $p<0.05$). The wound also had a probability of higher cumulative category and was positively associated with bacteria isolates ($\beta=0.271$, $df=1$, $p<0.05$)

From Figure 3, it is clear that the Multiple Antibiotic Resistance index (MAR) index that was greater than 0.2 (>0.2) for each of the bacteria isolates are 100%, 94.1% and 89.2% for *P. aeruginosa*, *E. coli* and *K. pneumoniae* respectively. MAR index values >0.2 is a reflection of isolates from high-risk contaminated source which is characterized by high frequency of antibiotics usage, this implied that patients whose profile were reported in this study have positive history of antibiotics use before their presentation to the clinic.

DISCUSSION

Antimicrobial resistance in enterobacteria which are also gram-negative such as *Klebsiella pneumoniae* and *E. coli* has been identified as a great peril to human health [21]. Recently, cases of antimicrobial resistance have been reported to be on the increase notably among *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter* species [4]. Infections caused by *Pseudomonas aeruginosa* are common in individuals with an immunocompromised state and patients with invasive devices [22], owing to their combination of intrinsic and acquired mechanisms, *P. aeruginosa* have been established to rapidly develop resistance to all effective antibiotics [23].

In a one-year retrospective study conducted in Kuwait, focusing on *E. coli*, *K. pneumoniae* and *P. aeruginosa* and *Acinetobacter baumannii*, the prevalence rate of was reported to be 51.9%, 21.3% and 15.7% for *E. coli*, *K. pneumoniae* and *P. aeruginosa* respectively [24], which are slightly lower than the respective prevalence rates of 59.8%, 29.0% and 11.2% obtained in this study. However, results from this study agrees with findings from a study involving only 2 of the isolates where *E. coli* was 54.5% and *K. pneumoniae* was 29% prevalent [25]. In a similar study that involved several clinical specimens, *E. coli* isolates was 68.5% and *K. pneumoniae* was 31.5% prevalent [26], which are higher values than the rates obtained in this study. Notably, prevalence rates by previous studies on *P. aeruginosa* does not align with findings from this present study. Prevalence rate of 75.7% was reported in the study on AMR in *P. aeruginosa* [27], while 54.16% prevalence rates of *P. aeruginosa* was reported in a study involving only wound specimen [28], and 41.9% and 39.4% was recorded respectively in the study that involved ear and wound swab specimen only [29].

The age group 21-30 and 31-40 were responsible for almost 60% of total isolates in this study, this aligned with findings in their study [30]. These age group are mostly in their reproductive age and sexually active, with majority of the specimen from this age group being from urine, semen and HVS which could have been responsible for the higher frequency gotten from them. However, there is never a strong reason for comparison between the age group owing to the fact that more adults were recruited into this study than children, hence, the observation that more isolates were obtained from adults corresponds with the high number of adult clients recorded.

The wet season was characterized by higher isolation rate (179) for the 3 bacteria in this study. Although, no previous study has documented this observation in the 3 bacteria in this study. Previous documentation has been for cases of *Vibrio cholerae*, another notifiable and bacteria of public health importance where there has been established relationship between rainy season and higher prevalence of cholera [31]. There has also been the establishment of the incidence of Community acquired pneumonia (CAP) being higher during the rainy season [32] but not all cases of CAP are caused by *K. pneumoniae*.

A higher frequency of isolates from urine were from the female, the reason for this could not be far-fetched from the anatomical position of the female urethra in close proximity to the anus which predisposes it to

invasion by both the skin and fecal flora. The bacteria, *E. coli* being the highest isolate from urine in this study, followed by *K. pneumoniae* and *P. aeruginosa* which corroborates the research findings earlier reported [33]. Bulk of *P. aeruginosa* were isolated from wound (31.3%) and urine (28.1%) samples, this is in agreement with the research report where majority of *P. aeruginosa* were isolated from wound (44%) and urine (30%) [34]; however, our findings sharply disagree with results from previous studies that documented in their respective studies that the highest frequency of *P. aeruginosa* was obtained from urine specimen [33], [35].

The multinominal (ordinal) regression analysis showed that the clinical specimen from which bacteria isolate were identified might have tendency to influence the resistance of such isolate to antibiotics. This is more of important to isolates from body fluids including semen, urine, HVS, blood and sputum. Age of patient is another factor that could positively and significantly influence the resistance or susceptibility of the organisms to drug action. More importantly, season of the years and patient gender had no significant relationship.

The antibiotics to which the 3 isolates altogether showed highest resistance to was Imipenem 159(55.6%) which belongs to the antibiotic class Carbapenem. Carbapenem has always been restricted for the treatment of resistant bacteria. In recent times, many hospitals have reported the spread of carbapenem resistance among the Enterobacteriaceae at an alarming rate globally, specifically within the *E. coli* and *K. pneumoniae* [36]. This is closely followed by Cefixime 157(54.9%), this does not agree with findings in the study conducted in Kuwait where antimicrobial prevalence rate was higher towards Cefotaxime, Ciprofloxacin and Cefuroxime; while resistance to Imipenem was lower [27]. This study findings also contrast the findings regarding resistance to carbapenem by the isolates in a multicenter study carried out in Kuwait and Nigeria where the rate of 14% and 8% were obtained respectively [25]. Resistance rates toward LBC (16.4%) and CIP (27.5%) in this study also affirmed the report from USA about the recent rise in fluoroquinolone resistance among the Enterobacteriaceae (*E. coli* and *K. pneumoniae*) [37].

Antimicrobial resistance rates by *E. coli* as obtained in this study are: LBC (16.4%), ZEM (58.5%), IMP (53.2%), CTX (52.6%), CXM (45.0%), CRO (22.2%), GEN (23.9%), AUG (50.9%) and CIP (27.5%). The increased rate of resistance to Imipenem, a carbapenem as found in this study is a confirmation of the rapid development of carbapenem-resistant *E. coli*, as reported in an earlier study [38]. This study however disagrees with findings of 0%, 100% and 100% resistance towards IMP, AUG and CTX reported in the study [25]. Contrast report of AMR rates of 100% (CTX), 96.5% (CIP), 80% (GEN) and 9.7% (IMP) was also reported in a study conducted in Lagos [39]. Findings from a study conducted in Netherland also showed a lower rate of resistance by *E. coli* with the frequencies; CIP (12%), AUG (11%), GEN (6%) and CXM (5%) [40]. The study with diverse clinical specimens as our study also have a different resistance rate from our study; resistance rates were reported to be; AUG (69%), GEN (54%), CRO (78%), CXM (89%) and IMP (0%) [26]. Reports from UK, Norway and Spain, shows that *E. coli* are highly susceptible to quinolones and cephalosporins [41], [42] which contrast findings from this study where higher rate of resistance to cephalosporins (CXM, CTX, ZEM) were recorded, although findings in this study also indicates that *E. coli* are still slightly susceptible to fluoroquinolones.

Resistance rates by *K. pneumoniae* in this study are; LBC 25.3%, ZEM 40.9%, IMP 57.8%, CTX 50.6%, CXM 57.8%, CRO 25.3%, GEN 20.5%, AUG 55.4% and CIP 34.9%. Resistance to IMP was higher from this study is contrary to the report of 8% resistance in a previous study [40], and slightly lower to resistance documented towards IMP (67%) observed in a study conducted in India [43]. The highest drug resistance in this present study was towards CXM and IMP (57.8%) which disagree with highest resistance recorded for GEN in another study [34].

Resistance rates by *P. aeruginosa* in this study are; LBC 25%, ZEM 71.9%, IMP 62.5%, CTX 56.25%, CXM 75%, CRO 31.3%, GEN 40.6%, AUG 50% and CIP 46.9%). The higher rates of resistance to cephalosporins

agrees with the reports from a multi-center survey conducted across southwestern Nigeria, where higher resistance to cephalosporins were also documented in their studies [44], [45]. Resistance to antimicrobial drugs was reported to be higher towards, Cefepime, Augmentin, Cefotaxime and Cefpodoxime. Same study also reported 29.36% of the total isolates from wound infections were *P. aeruginosa*, and all the isolates were reported to be multidrug resistant [45]. In the study conducted in a tertiary health facility in Maiduguri, resistance rates towards IMP, GEN and CIP were reported to be 100%, 45%, 22% respectively [46]. These values are higher than the findings from this present report except for resistance towards CIP which was lower. The 100% resistance rate to IMP reported in Maiduguri and 65% from this study is an indication that carbapenem resistance is rapidly evolving in Nigeria.

The high Multidrug-resistance rate (MDR) recorded in this study is quite disturbing. Overall Multidrug-resistance rate (MDR) prevalence rate in this study is very high with the value of 68.5% which does not agree with the MDR rate of 38.7% reported from a study in Kuwait [27]. The individualized MDR rates from this study are; 66.1%, 71.1% and 75% for *E. coli*, *K. pneumoniae* and *P. aeruginosa* respectively. This MDR rate contrasts reports from other studies for *K. pneumoniae*; 89%, and 91.7% [39], [47] which are higher than the rate obtained in this study and 37.7% reported in another study [48] which is lower than the MDR report from this present study. This report is however in alignment with the MDR reported by researches conducted in some hospitals in Lagos, where high MDR strains of *K. pneumoniae* was also reported [49]. Also, MDR rate for *P. aeruginosa* obtained in this study is 75%, previous studies reported MDR rate of 12.8% and 19.6% respectively in previous researches [44], [45] which are both lower than the rate obtained in this study, this implies there is rapid development of AMR within the *Pseudomonas aeruginosa*. In 2017, multidrug-resistant *Pseudomonas aeruginosa* was responsible for an estimated 32,600 infections among hospitalized patients and 2,700 estimated mortalities [50].

The high MAR Index recorded in this study corroborates the findings from MAAP survey where the Drug Resistance Index (DRI) for Nigeria was reported to be 65.90% [19]. In previous studies, high MAR Index has been documented to be associated with the high frequency of antibiotics use among human population especially in resource-poor settings [51]. In community settings, over-the-counter access to drugs is common, thus aiding the accessibility to broader range of antibiotics either through inappropriate prescription, self-medication which is a common practice in low- and middle-income countries (LMICs) [52].

48 diverse MDR profiles were observed within the 113 MDR *E. coli* isolates in this study which implies 42.5% diversity, this differs from the MDR profiles of 23.7% among 59 MDR *E. coli* isolates as reported in another study [30]. For *P. aeruginosa*, 13 MDR profiles were observed which represents 54.2% diversity. 27 diverse MDR phenotypes were obtained for *K. pneumoniae*, which shows 45.8% diversity among the isolates. Both quinolones and cephalosporins occurred in 50% of MDR profiles generated for *E. coli* in this study which does not align with 64.29% documented in a previous study [53].

The increasing rate of resistance to Imipenem, a carbapenem as found in this study is a confirmation of the rapid development of carbapenem-resistant *E. coli* as documented in an earlier survey [38]. Resistance rate recorded for fluoroquinolones (LBC and CIP) also affirmed the report from USA about the recent rise in fluoroquinolone resistance especially among the Enterobacteriaceae [37]. Susceptibilities to quinolones and cephalosporins by *E. coli* as documented in previous studies [41], [42], [54] are reportedly higher than what is observed in this study. Findings from this study shows that *E. coli* is highly resistant to cephalosporins, although findings in this study also indicates that *E. coli* are highly susceptible to quinolones.

CONCLUSION

This study aligned with the One-Health surveillance for antimicrobial resistance (AMR), with focus on the

human interphase. The high susceptibility rate observed towards gentamycin (GEN) is a function of its availability option as only a parenteral antibiotic, thus clinical and community abuse is minimal and this must be maintained due to its relevance in the management of urinary tract infections. The higher rate of resistance to (cephalosporin) cefuroxime, cefixime, cefotaxime and ceftriaxone as demonstrated by the three (3) bacterial isolates is significantly above average, except for ceftriaxone where resistance was 24.1%. These antibiotics have been identified by WHO to be critically important in the management of bacterial infections. Cephalosporins (CTX, CXM, CRO, ZEM) are drugs of choice in the management of severe infections caused by *Pseudomonas aeruginosa*, the higher resistance rates to these drugs as observed in this study is an indication of overuse in the hospital settings, hence it cannot be totally alienated from being the risk factor in the spike in the development of resistance against this class of antibiotics by the organism.

The overall higher level of resistance to all the drugs observed from this study could be linked with indiscriminate use of drugs, misuse of drugs and lack of appropriate and adequate laws and policies regarding the sale and purchase of antibiotics. Carbapenem have always been used as last resort for its potency against arrays of infections caused by Gram-negative bacteria, but findings from this study revealed an above average resistance rate which is quite worrisome understanding how difficult it could get in managing infections by ESBL-producing bacteria. The great diversity in the MDR profiles as expressed by the 3 bacterial isolates is also of great concern as it could be inferred that several drug-combination therapies would always be required in the management of infections caused by any of these bacteria either within the hospital settings or as community-acquired infections. This would pose a great hazard to our not so-good performing health care system as emerging and re-emerging bacterial infections might be difficult to halt.

RECOMMENDATIONS

Surveillance, education and awareness of antimicrobial stewardship aligns with the goals of public health to promote health, prolong life and prevent diseases. The very high resistance rate and extremely high MAR Index as well as the extremely diverse MDR profile in this study predicts an inevitable disaster at the community level, thus necessitating the need for continuous monitoring and implementation of effective policies on antimicrobial use, adequate antimicrobial sale and purchase regulations. Public health professionals need to organize campaigns on the dangers that indiscriminate use of antibiotics pose in the development of AMR. Antimicrobial stewardship clubs should be established in schools as this would help to create the consciousness on the need to use antibiotics with caution among the younger generation which would help in forming their future attitude and perspectives towards misuse and inappropriate use of antibiotics. There is also the need for a more comprehensive and possibly state-wide surveillance to ascertain the present trend and pattern of antimicrobial resistance as this would guide in healthcare practices regarding antibiotics prescription, adequate diagnostic procedures before antibiotics prescription, as well as good antimicrobial stewardship practices across all boards.

Ethical Consideration

A formal application for ethical clearance was obtained from Babcock University Research and Health Ethics Committee (BURHEC). Approval was granted with the assigned number; BURHEC 1019/23. Same was presented to the director of the health facility and his approval was gotten. Secondary data were made use of for this study, hence there was no need for informed consent to be sought. However, all data from the healthcare facility were treated with confidentiality and information gotten were strictly used for the purpose for which it was collected, no third party had access to the raw data. Also, the researcher ensured that there were no bias in the data presentation, assessment and interpretation of results.

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Conflict of Interest

The authors declare that they have no competing interests with respect to the study, authorship and publication of this article.

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Authors Contribution

Conceptualization, data analysis and original draft OO. Revision and editing- OO and TO. All authors have read and approved the manuscript.

Tables

Table 1: Age group and Gender Distribution

Age Group	Male	Female	Percentage (%)
0.1-10	3	7	10 (3.5%)
11-20	6	6	12 (4.2%)
21-30	23	53	76 (26.6%)
31-40	55	39	94 (32.9%)
41-50	30	17	47 (16.4%)
51-60	13	4	17 (5.9%)
61-70	9	4	13 (4.5%)
71-80	11	2	13 (4.5%)
81-90	3	0	3 (1.0%)
91-100	1	0	1 (0.3%)
TOTAL (286)	154	132	286 (100%)

Age and gender relationship with respect to the study.

Table 2: Clinical Specimens and Isolate Distribution

Clinical Specimens	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	Total
Blood	0	8	7	15
Ear swab	0	4	3	7
ECS	4	0	0	4
Semen	59	9	1	69
Sputum	1	18	0	19
Stool	3	0	0	3
UCS	7	1	0	8
Urine	47	13	9	69
VS	50	14	2	66
Wound	0	16	10	26
Total	171	83	32	286

Bacteria isolates distribution with respect to the clinical specimens.

Table 3: Months of the year and Isolates Distribution

Months	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
January	20	9	3
February	12	4	3
March	8	10	2
April	6	7	2
May	12	6	2
June	13	14	2
July	19	5	1
August	23	7	6
September	20	7	1
October	18	4	4
November	12	8	2
December	8	2	4
Total	171	83	32

Season was categorized into Wet and Dry season which was defined as; Wet season (April-October) and Dry season (November-March).

Table 4: Bacteria Isolates, resistance and susceptibility to individual antibiotics

Isolates	LBC	ZEM	IMP	CTX	CXM	CRO	GEN	AUG	CIP
<i>E. coli</i>	28	100	91	90	77	38	41	87	47
<i>K. pneumoniae</i>	21	34	48	42	48	21	17	46	29
<i>P. aeruginosa</i>	8	23	20	18	24	10	13	16	15
Total resistance	57	157	159	150	149	69	71	149	94
Susceptibility	128	97	96	85	103	154	171	87	157

Resistance was higher for the cephalosporin class (ZEM, CTX and CXM), Carbapenem (IMP).

Table 5: Bacterial Isolates and Resistance pattern

Isolates	No resistance	single resistance	Double resistance	MDR	Total
<i>Escherichia coli</i>	10	15	33	113	171
<i>Klebsiella pneumoniae</i>	9	3	12	59	83
<i>Pseudomonas aeruginosa</i>	0	1	7	24	32

MDR rate was notably high for the 3 isolates. There was no isolate of *Pseudomonas aeruginosa* that did not exhibit resistance to at least one of the antibiotics

Table 6: Multidrug resistance pattern/drug-pathogen relationship

Specimen	<i>E. coli</i> and Resistance pattern	<i>P. aeruginosa</i> and resistance Pattern	<i>K. pneumoniae</i> and resistance pattern
SEMEN	1). AUG-CTX-CXM-IMP-ZEM 2). AUG-CTX-CXM-ZEM 3). CRO-CTX-IMP-ZEM 4). CTX-IMP-ZEM 5). AUG-CIP-CTX-ZEM 6). AUG-CTX-CXM-GEN-IMP-ZEM 7). AUG-CTX-CXM-IMP 8). CIP-CTX-CXM-IMP-ZEM	No pattern, only 1 isolate from this specimen	1). AUG-CIP-CTX-CXM-GEN-IMP-ZEM 2). AUG-GEN-IMP-LBC-ZEM
BLOOD	No <i>E. coli</i> was isolated	1). CTX-CXM-GEN	1). AUG-CIP-CRO-CTX-CXM
URINE	1). AUG-IMP-ZEM 2). AUG-CIP-CXM-GEN-LBC-ZEM	1). CIP-CTX-IMP-ZEM 2). AUG-CIP-CRO-CTX-CXM-GEN-IMP-LBC-ZEM	1). AUG-CIP-CXM-IMP 2). CIP-CRO-CTX-CXM-GEN-IMP

	3). AUG-CRO-CTX-IMP-ZEM 4). CIP-IMP-ZEM 5). AUG-CTX-GEN-IMP 6). AUG-CTX-CXM-IMP-ZEM		
V SWAB	1). AUG-CTX-IMP-ZEM 2). AUG-CTX-CXM-IMP 3). AUG-CTX-IMP 4). AUG-CXM-CTX-GEN-IMP-ZEM 5). AUG-CTX-CXM-IMP-ZEM 6). AUG-CIP-CRO-CTX-CXM-GEN-IMP-LBC-ZEM 7). AUG-CTX-CXM-ZEM 8). CRO-CTX-CXM-GEN-ZEM	No pattern, only 2 isolates from this specimen	1). CTX-CXM-IMP-LBC-ZEM 2). AUG-CRO-CTX-GEN
ECS	1). CIP-IMP-ZEM	No <i>P. aeruginosa</i> was isolated	No <i>K. pneumoniae</i> was isolated
UCS	1). AUG-CTX-CXM-IMP-ZEM	No <i>P. aeruginosa</i> was isolated	No pattern, only 1 isolate from this specimen
EAR SWAB	No <i>E. coli</i> was isolated	No pattern, only 3 isolates from this specimen	1). CTX-CXM-IMP-ZEM
WOUND	No <i>E. coli</i> was isolated	1). AUG-CRO-CTX-CXM-ZEM 2). AUG-CIP-CRO-CTX-CXM-GEN-IMP-LBC-ZEM	1). CTX-CXM-IMP-LBC 2). AUG-CTX-CXM-ZEM 3). AUG-CIP-CRO-CTX-IMP-ZEM
STOOL	No pattern, only 3 samples were cultured	No <i>P. aeruginosa</i> was isolated	No <i>K. pneumoniae</i> was isolated
SPUTUM	No pattern, only 1 isolate of <i>E. coli</i> was from this specimen	No <i>P. aeruginosa</i> was isolated	1). AUG-CTX-CXM-IMP 2). AUG-IMP-ZEM 3). AUG-CIP-CRO-CTX-IMP-ZEM

N.B: Each pattern in this table has occurrence in 3 or more isolates of the bacteria where the resistance pattern was generated.

Table 7: Parameter estimates of ordinal regression model of factors influencing identified bacteria

Variable	Estimate	Std. Error	Wald	df	P-value	95% Confidence Interval (Lower)	95% Confidence Interval (Upper)
Threshold							
Constant = <i>E. coli</i>	17.881	1.414	159.9	1	0	15.11	20.653

Constant = <i>K. pneumoniae</i>	20.941	1.37	233.729	1	0	18.256	23.626
Independent Variables							
Gender	0.341	0.384	0.791	1	0.374	-0.411	1.093
Seasons	-0.442	0.303	2.134	1	0.144	-1.035	0.151
Age group	0.271	0.096	7.952	1	0.005	0.083	0.459
Semen	16.726	0.994	282.934	1	0	14.777	18.675
Urine	18.397	0.949	375.669	1	0	16.536	20.257
Blood	22.123	1.003	486.057	1	0	20.157	24.09
Sputum	19.814	1.035	366.306	1	0	17.785	21.843
Vswab	18.036	0.956	355.644	1	0	16.162	19.911
ECS	-0.403	6780.077	0	1	1	-13289.1	13288.3
UCB	16.966	1.422	142.427	1	0	14.18	19.753
Wound	21.319	0.956	497.006	1	0	19.445	23.193
Ear Swab	22.443	0	N/A	1	N/A	22.443	22.443
Stool	0a	N/A	N/A	0	N/A	N/A	N/A

a. This parameter is set to zero because it is redundant. na. this value is unavailable

Table 7: Ordinal regression model *P. aeruginosa* has lower frequency, hence the variable was latent

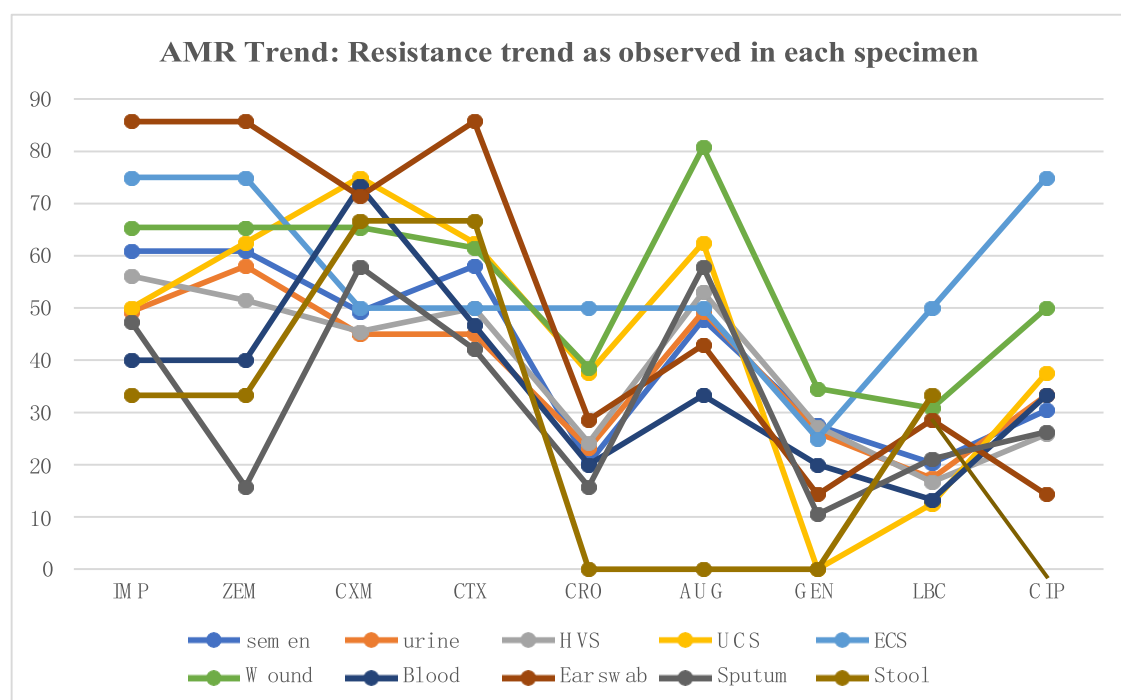


Figure 1: AMR trend within the clinical specimens

Bacteria isolated from stool demonstrated the least resistance to all the drugs while despite the low frequency, isolates from ECS was resistant to all the drugs at 50% or greater except towards GEN which was common to all other clinical specimens.

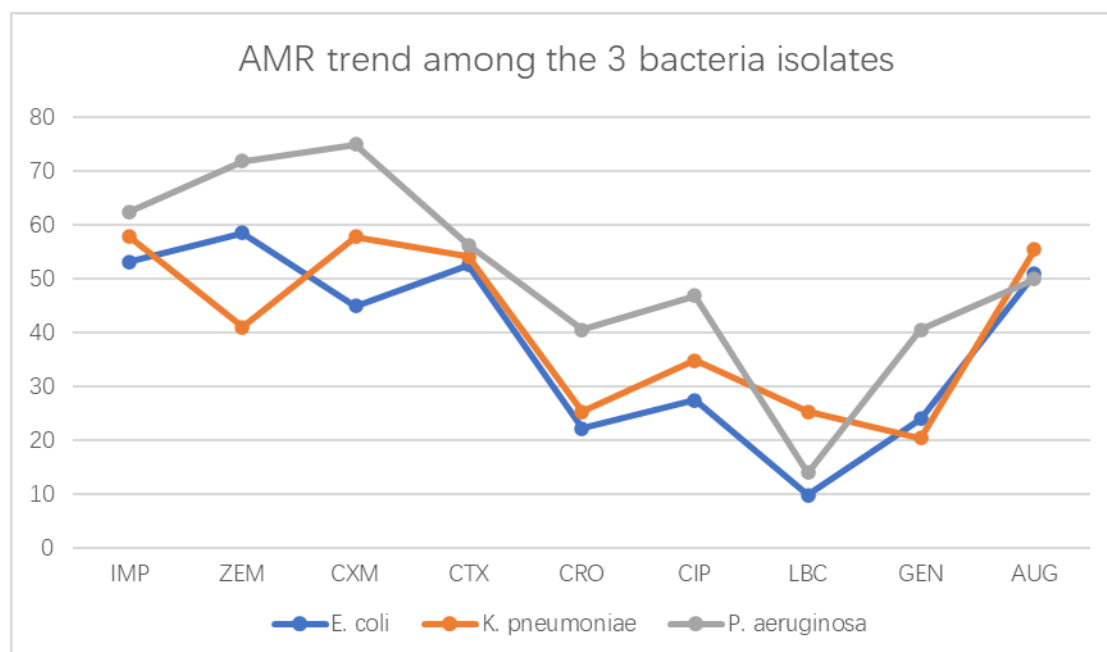


Figure 2: AMR Trend in *E. coli*, *K. pneumoniae* and *P. aeruginosa*

Among the 3 bacterial isolates, *P. aeruginosa* demonstrated the highest resistance despite having the lowest frequency. The trio demonstrated similar pattern towards almost all the tested antibiotics i.e it is almost always either all-high or all-low for each particular drug.

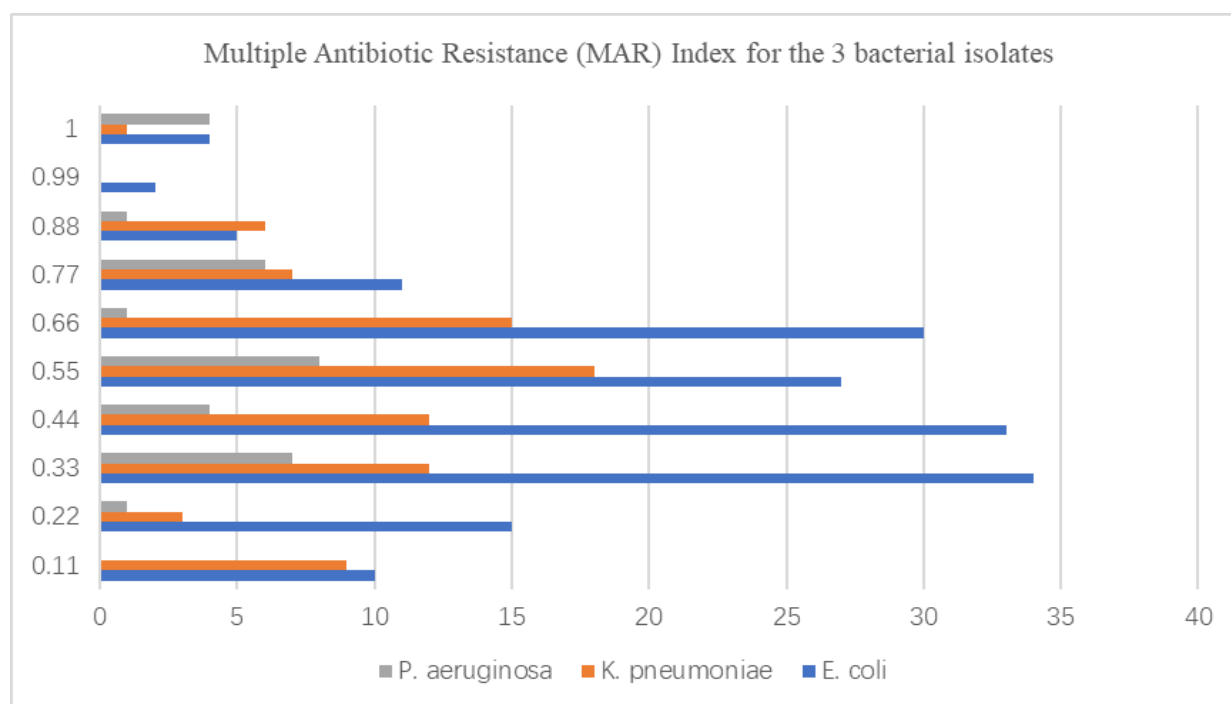


Figure 3: Multiple Antibiotic Resistance (MAR) Index for *E. coli*, *K. pneumoniae* and *P. aeruginosa*

(MAR index = number of antibiotics resistant to / total number of antibiotics tested). MAR index values > 0.2 indicate existence of isolate from high-risk contaminated source with frequency use of antibiotics while values

≤ 0.2 show bacteria from source with less antibiotic's usage.

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