Original Article

Multiple Antibiotic Resistance Index of *EscherichiaColi* Isolates in a Tertiary Hospital in South-West Nigeria

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ABSTRACT

Background: The effectiveness of available antibiotics is reducing as microorganisms device means of evading its effects, resulting in the development of superbugs. Pathogens previously susceptible are becoming resistant, and spreading beyond the hospital environment. This change is a major concern for infection control and prevention and a huge economic burden for health care. This study aims to determine the drug sensitivity pattern of E. coli isolated in a tertiary hospital lab

Methodology: Gram negative bacilli, lactose fermenter, motile, indole positive, glucose fermenter, gas producing isolate were identified as E.coli. Antimicrobial susceptibility testing to commonly prescribed antibiotics was carried out using the modified Kirby Bauer method and reported with the Clinical and Laboratory Standard Institute Interpretative chart. Mulitiple Antibiotic Resistance index was calculated as number of antibiotics to which isolate is resistant divided by the total number of antibiotics against which isolate was tested.

Result: Five hundred and twenty–seven isolates were identified, among which a high rate of resistance to cefuroxime (407), high rate of intermediate susceptibility to nalidixic acid (264) and high rate of sensitivity to ceftriaxone (388) was noted. Sixty percent of the isolates had an index $\leqslant 0.2$. Of the 40% with Multiple Antibiotic Resistance index > 0.2, 61 % were from inpatient samples.

Conclusion: The high Multiple Antibiotic Resistance index of the E. coli isolates indicates previous exposure to antibiotics and development of resistance to commonly prescribed antibiotics, hence, antimicrobial susceptibility testing is imperative in selecting therapeutic options. Attention also needs be paid to effective infection control and prevention to curb its spread among individuals.

INTRODUCTION

Morbidity and mortality in low income countries, most of which are found in Africa, is from Group I conditions which includes infections, maternal, perinatal and nutritional conditions.[1] Useful available medications in the treatment of infections is declining as a result of increasing failure rate as the infectious agents device means of evading the effects of these drugs. In a global review on antimicrobial resistance, it was estimated that annual

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deaths attributable to antibiotic-resistant infections is about 700,000 and is likely to rise to about 10 Million by 2050 if action is not taken to combat this condition whose effect is especially felt in countries collectively referred to as the BRIC (Brazil, Russia, India, China) and MINT (Mexico, Indonesia, Nigeria, Turkey) world.[2]

The development of superbugs could result in increased mortality from infections and can spread to the larger population. The spread will further worsens economic and manpower loss. The resultant effect of extensive uses of antimicrobial agents and development of superbugs include the shift in hospital acquired pathogens from easily treatable towards more resistant organisms and these resistant organisms' spread is no longer limited to the hospital environment. Multiply drug resistant community acquired infections are now being reported, the frequency of which is on the rise. This change is a major concern for nosocomial infection control and prevention and a huge economic burden for health care as the cost of care for patients with resistant infections is higher than for patients with non-resistant infections due to longer duration of illnesses, additional tests and use of more expensive drugs.[3,4]

The Center for Disease Control (CDC) in 2013 published a report outlining the top 18 drug-resistant threats, categorized based on the level of concern into urgent, serious, and concerning. Urgent threats have the potential to become widespread but are not currently widespread, hence require urgent public health attention to identify the infections and limit its transmission. Examples of organisms in this category are *C.difficile*, carbepenem resistant Enterobacteriacea and drug resistant *Neisseria gonorrheae*. Serious threat category are those that may worsen and become urgent without ongoing public health monitoring and prevention activities. Examples of organisms in this category include the extended spectrum betalactamase (ESBL) producing enterobacteriaceae. Concerning threat category have lesser threat of antibiotic resistance and there are multiple therapeutic options for resistant infections. Examples of organisms in this category include the Vancomycin resistant *Staphylococcus aureus* (VRSA), erythromycin resistant Group A Streptococcus, clindamycin resistant Grp B Streptococcus.[5]

Drug resistant *E.coli* falls into the serious threat category. Urgent and serious categories require more monitoring and prevention activities, hence the need to closely monitor the *E.coli* isolated from our lab by determining the likelihood of it being resistant to commonly prescribed antibiotics, and the source of the isolate with the aim of curbing its spread and transmission.

Escherichia coli is a gram negative motile facultatively anaerobic bacilli which may or may not be encapsulated.[6] It causes a wide range of infections in humans and it is most commonly implicated in urinary tract infection. It is one of the commonly isolated organisms in the clinical microbiology laboratory in this environment possibly due to its nonfastidious nature.[6] It is found naturally in the gut of warm blooded animals, man inclusive hence, when isolated in food or water, it is an indication of the presence of enteric pathogens or faecal contamination. Its dissemination throughout the environment is via faeces of man and animals. This, coupled with the ability of the bacterium to survive for months external to the colon, makes the bacterium almost ubiquitous.[6]

E.coli isolate which is a normal flora of the gut differs from *E.coli* that has been adapted to survive outside the gut, as contaminants in food and water. A procedure which would distinguish between *E. coli* originating from high-risk environments and *E. coli* originating from other sources would provide a quantum of definition not possible with current laboratory procedures. Indexing *E. coli* isolates obtained from food according to the frequency with which multiple antibiotic resistances (MAR) occur may provide a relatively easy method for making this distinction.[7]

Antimicrobial resistance is a natural occurrence in microorganisms, occurring naturally over time, usually through several means like genetic changes,

drug efflux, down regulation of receptors, secretion of inactivating enzymes such as beta lactamases which is the mechanism of resistance in *E.coli* and other members of the Enterobacteriaceae family. It is a means of adaptation for survival in the organism. Two principal drivers of resistance appear to be inadequate (or inappropriate) empirical antibiotic therapy and prolonged antibiotic use.[8,9]

Misuse and overuse of antimicrobials however is accelerating this process. In many places, antibiotics are overused and misused in people and animals, as it is often taken without prescription by a professional, used for cases not proven to be of bacterial cause and included in animal feeds as growth promoters.[4]

It has been demonstrated that the sub-therapeutic use of antibiotics in the mass production of poultry, eggs, and pork has promoted the emergence of and maintains the prevalence of MAR *E. coli* in the faecal environment of these animals. The wide use and abuse of antibiotics in human therapy has produced MAR *E. coli* in the faeces of humans as well. These practices have resulted in the co-existence of MAR *E.coli* within these major reservoirs of enteric disease for humans.[7,19,20]

Likewise, the introduction of new antibiotics has not kept pace with the increasing rate of re-sistance, leaving clinicians with fewer treatment options. A survey carried out in the United States showed that of the 506 new drugs in development, only 5 were antibiotics and reports shows that the pharmaceutical pipeline for new antibiotics are drying up.[10]These antimicrobial resistant-microbes are not limited to the hospital environment but have also been found in people, animals, food, and the environment outside the hospital. They can spread between people and animals, and from person to person. Poor infection control, inadequate sanitary conditions and inappropriate food-handling encourage the spread of antimicrobial resistance.[4]

The extended spectrum beta-lactamase (ESBL) producing bacteria especially the gram negative in the family Enterobacteriaceae are found commonly in the community and hospital environment, and are becoming associated with clinical and treatment failure.[11] This form of resistance have been reported in several classes of bacterial with multi-drug resistant *Klebsiella* species and *E. coli* been isolated in hospitals around the world.[12,15] Even in Nigeria, several cases of multidrug resistant gram negative bacteria have been isolated from different clinical samples such as Multidrug Resistant (MDR) *Klebsiellapneumponiae*[13,14,15],MDR Enterobacter spp.[15,16] MDR *E.coli*[17] to mention but a few. In a study carried out by Chijioke et al where *E.coli* isolated from five geopolitical zones of Nigeria were screened for anti-microbial resistance, it was reported that a total of 42 different antibiotics resistance profiles were seen, with all the isolates showing resistance to at least four or more of the drugs tested.[17]

With increasing resistance to existing antibiotics, developing countries face a serious challenge in safeguarding their populations' health.[18] This identified challenge, and the frequency of isolating *E.coli* in our laboratory combined with the serious threat category in which MDR *E.coli* falls makes this study imperative, to determine the propensity of the *E.coli* isolate in our laboratory being from a high risk source hence drug resistant while determining the pattern of resistance especially to affordable, available and commonly prescribed antimicrobial agents. We hereby retrospectively reviewed the reported pattern of susceptibility to antibiotics with the aim of determining the presence of multiple drug resistant strains in our environment, advice on preventing its development and curbing its spread if present as well as determine the preferred antibiotics for the treatment of infections due to this isolates.

MATERIALS AND METHODS

A retrospective analysis of all *E.coli* isolated from the hospital laboratory within a 26 months review period between January 2014 and February 2016 was carried out. The study was carried out at the Medical microbiology laboratory of Ekiti State University Teaching Hospital (EKSUTH), Ado-Ekiti, Nigeria. The hospital which use to be a general hospital was recently upgraded to a teaching hospital

status in 2008 and serves as a referral center to other secondary and primary health institutions in Ekiti state and its neighboring states of Osun, Ondo, Oyo, Kogi and Kwara states.

A combination of colonial morphological characteristics, gram stain reaction and biochemical analysis carried out on lactose fermenting growth was used in identification. Gram negative bacilli, motile, indole positive, glucose fermentation with gas production on Triple sugar Iron (TSI) slant, were presumptively identified as *E.coli*. Antimicrobial susceptibility testing was done using the modified Kirby Bauer method of disc diffusion and reported as Susceptible, Intermediate or Resistant comparing with the CLSI standard. Antibiotics tested were Gentamycin, Ofloxacin, Ciprofloxacin, Augmentin, Ceftazidime, Ceftriaxone, Cefixime, Cefuroxime, Nitrofurantoin, Cotrimoxazole, Nalidixic acid, Levofloxacin, and Tetracycline. *Escherichia coli* ATCC25922 was used as control strain. The MAR index was calculated following the prescription of Krumperman as number of antibiotics to which isolate is resistant divided by the total number of antibiotics against which isolate was tested.

RESULTS

A total of 527 isolates identified by colonial morphology and biochemical tests as Escherichia coli were recovered from various clinical samples within the review period. 59.8% were recovered from urine sample while least recovery was from Cerebrospinal Fluid (0.4%). (Table 1)

Table 1: Distribution of *E. coli* by clinical sample and sex

Sample	Male (n%)	Female (n%)	Totaln (%)	χ^2	*p* value
Urine	138 (62.4)	177 (57.8)	315 (59.8)		
Sputum	9 (4.1)	8 (2.6)	17 (3.2)		
Swab	53 (24.0)	112 (36.6)	165 (31.3)		
Semen	8 (3.6)	-	8 (1.5)		
Stool	7 (3.2)	6 (2.0)	13 (2.5)		
Blood	5 (2.3)	2 (0.7)	7 (1.3)		
C.S.F.	1 (0.4)	1 (0.3)	2 (0.4)		
Total	**221 (100.0)**	**306 (100.0)**	**527 (100.0)**	22.215	0.001*

CSF- Cerebrospinal fluid

The isolates' susceptibility to commonly prescribed antibiotics in the hospital was determined using the modified Kirby Bauer method (Table2). A MDR rate of 60.2% was noted as 317 isolates were found to be resistant to at least one agent in three or more antimicrobial categories According to the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) definition of MDR.

Table 2: Antibiotic sensitivity pattern of *E coli* from various clinical specimen.

Antibiotic (Concentration in disc)	N	Sensitive (%)	Intermediate (%)	Resistant (%)
Gentamycin(10µg)	527	299(56.7)	0(0)	228(43.3)
Ofloxacin(5µg)	527	224(42.5)	0(0)	303(57.5)
Ciprofloxacin (5µg)	527	260(49.3)	0(0)	267(50.7)
Augmentin (20/10µg)	527	160(30.4)	42(8)	325(51.7)
Ceftazidime (30?g)	527	255(48.4)	0(0)	272(51.6)
Ceftriaxone(30?g)	527	388**(73.6)**	66(12.5)	73(13.9)
Cefixime (5µg)	527	98(31.1)	170(32.3)	259(49.1)
Cefuroxime (30µg)	527	108(20.5)	12(2.3)	407(77.2)
Nitrofurantoin (300µg)	315	263(83.5)	0(0)	52(16.5)
Cotrimoxazole (5µg)	332	128(38.6)	104(31.3)	100(30.1)
Nalidixic acid (30µg)	315	42(13.3)	264(83.8)	9(2.9)
Levofloxacin (1µg)	123	66(53.7)	50(40.7)	7(5.7)
Tetracycline(30 µg)	13	4(30.8)	0(0)	9(69.2)

A high rate of resistance to cefuroxime was noted (407), high rate of intermediate susceptibility to nalidixicacid(264) and high rate of sensitivity to ceftriaxone(388) was noted.

When the MAR indices of the isolates was calculated, 60% were found to have an index $\leqslant 0.2$ while none had an index of 1.0, being resistant to all antibiotics it was tested against .(Table 3, figure 1). Of the 40% of the total isolate with MAR index > 0.2, 61 % of it were isolated from clinical samples collected from inpatients on admission at the hospital.

Table 3: Multiple Antibiotic Resistance (MAR) Index of Isolates

MAR index	No of isolates (%)
0.1	109(20.7)
0.2	101(19.2)
0.3	61(11.6)
0.4	34(6.5)
0.5	90(17.1)
0.6	29(5.5)
0.7	9(1.7)
0.8	79(15.0)
0.9	15(2.8)
1.0	0(0)

Figure 1: Distribution of MAR index

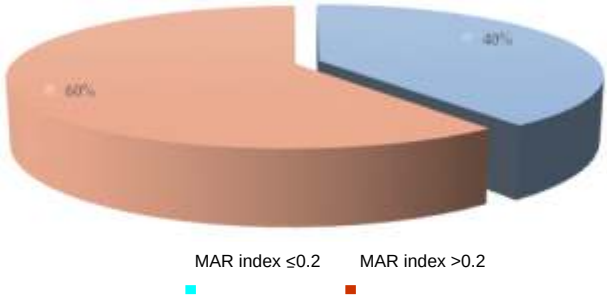
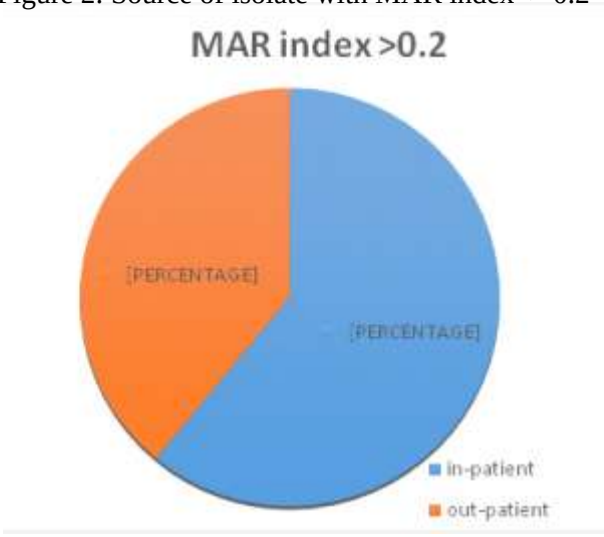


Figure 2: Source of isolate with MAR index > 0.2



DISCUSSION

Multiple antibiotic resistance index is helpful in analyzing health risk, as well as to check the extent of antibiotic resistance.[21] MAR index analysis has been used to differentiate isolates from different sources using antibiotics that are commonly used in treatment of infectious cases. Compared to other methods of bacteria source tracking, it is cost effective, rapid, easy to perform, does not require special training and expensive equipments.[22] MAR indexing has been found to be a simpler and cheaper means of identifying sources of isolate when compared to the established molecular diagnostic methods.[23] A MAR index value greater than 0.2 indicate high risk source of contamination where antibiotics are often used.[22]

A significant number of *E.coli* isolate from this study had a MAR index > 0.2 indicating their source to be from sources where antibiotics are commonly used, or previous exposure of the organism to antimicrobial agents. In order words, isolates are from high risk sources of antibiotic resistance. These isolates were from both inpatients and outpatients, pointer to the fact that antibiotic resistance and development of superbugs is not limited to hospital acquired pathogens only but can be from community acquired pathogens too.

The menace of antimicrobial resistance is particularly worrisome in developing countries like ours where there is a high burden of infectious disease with concomitant high rate of poverty which constrains the access to newer, more effective and conversely more expensive antimicrobial agents.[24] Multidrug resistance, also referred to as Multiple Drug Resistance (MDR) can be defined as resistance of a microorganism to multiple antimicrobial drugs. It was defined by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), as non-susceptibility to at least one agent in three or more anti-microbial categories.[25]Of particular public health importance is the MDR bacteria strains for it is commoner in occurrence. Isolated more often are Vancomycin-Resistant Enterococci (VRE), Methicillin-Resistant Staphylococcus aureus

(MRSA), Extended-spectrum β-lactamase (ESBLs) producing Gram-negative bacteria, Multidrug-Resistant gram negative rods (MDR GNR) such as *Enterobacter* species, *E.coli*, *Klebsiellapneumoniae*, *Acinetobacterbaumannii*, and *Pseudomonasaeruginosa*.[26] MDR pathogens are widespread in our environment due to the indiscriminate use of antibiotics which could be easily procured over the counter, without a proper investigation and physician's prescription. This results in clearing off of susceptible organisms leaving the drug resistant ones to be transmitted and circulated from person to person.[27,28,29] In the same vein, the exposure of organisms to antibiotics, either in human or in animal feeds stimulate mutation in the organism, making them resistant to the commonly used antimicrobial agents. This trend is what is seen in this study with a high rate of resistance reported to commonly prescribed antibiotics used to treat common infections like cellulitis, urinary tract infection, respiratory tract infection, and other mild cases of infectious aetiology for which the populace present to the hospital for treatment. More than half of the isolate tested were found to be resistant to commonly used Fluoroquinolones such as like ciprofloxacin (57.5%), Amoxicicil-lin/clavulanatec ombination(51.7%),oral cephalosporin like Cefuroxime(77.2%), and Tetracycline(69.2%) and a significant number reported intermediate susceptibility to Cotrimoxazole(31.3%) and Nalidixic acid(83.8%). This high level of resistance is similar to the earlier reports of David et al in which an emergence of β lactamases especially cephalosporinases have been reported in Kenya and Nigeria.[30] Likewise, a study carried out in at Sudan reported a high resistance to Amoxicillin, Cefuroxime, Trimethoprim-sulfamethoxazole, Tetracycline and the fluoroquinoloneofloxacin and ciprofloxacin.[12] This can be due to inappropriate use of the concerned antibiotics. The MDR strains were also noted to be ESBL producers which likely explains the resistance to cephalosporins. The reported resistant patterns could also be due to known acquired resistance genes.[12,31]

Another study by Okeke et al in Lagos on isolates from Nigerian students found five drugs for which a considerable rise in resistance was seen during the period of review. These were Ampicillin, Sulfonamides, Streptomycin, Chloramphenicol, and Tetracycline. A common characteristic to them being their wide use, availability and affordability in Nigeria and other developing countries.[32] A moderate level of resistance to Trimethoprim (47.6%) and low level to Nalidixic acid (3.2%) was also noted in their study. These five inexpensive drugs are widely available without prescription from authorized health institutions and pharmacies, as well as from unauthorized patent medicine shops. The frequency of use of Fluoroquilonones and Nalidixic acid is on the rise nowadays hence the trend of low resistance to these agents as previously reported in earlier surveys and corroborated by our finding in this review may be altered with time.[6,24]Okeke et al further noted a rapid increase in the prevalence of resistance in commensal *E. coli* to most of the older, less expensive antimicrobial drugs used in the management of infections in Nigeria and opined that these strains are potential causes of infection as well as being potential reservoirs of resistance genes that could be transferred to other bac-teria.[6]

CONCLUSION

This study concluded that *E. coli* in the study environment has the propensity of being multidrug resistant as many commonly prescribed antibiotics are no longer effective against it. A significant number of *E.coli* isolate had a MAR index > 0.2 indicating their source to be from high risk sources, being previously exposed to antibiotics. The usefulness of many of the antibiotics in current use in clinical practice is declining with the high rate and yet increasing trends of resistance to them. Nitrofurantoin, Ceftriaxone, Nalidixic acid, and possibly Levoflaxacin may still be of benefit in therapeutic interventions in cases where *E.coli* has been implicated in our locality. A proactive measure to curb these menace of antibiotic resistance in this organism is urgently required to conserve the usefulness of the few drugs to which low or no resistance has been reported.

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