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Distribution of Multiple Antibiotic Resistance Index in a Setting where Fluoroquinolones and β-Lactams are used Arbitrarily.

Helen Oroboghae Ogefere[1], Imuentiyan Sylvia Osayande[1], Ephraim Ehidiamen Ibadin[2] and Richard Omoregie[1,2,*]

Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria [1], Medical Microbiology Division, Medical Laboratory Services, University of Benin Teaching Hospital, Benin City, Nigeria [2].

Author for Correspondence*: richard.omoregie@ubth.org

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Abstract

Antimicrobial resistance is a public health issue globally. Unregulated use of antimicrobials is rife in our setting, and multiple antibiotic (MAR) index is an inexpensive surveillance tool to monitor antimicrobial resistance in an environment. The study aimed to determine the MAR index among Gram negative bacilli from various locations in a tertiary hospital as well as the effect of concurrent ESBL production and ciprofloxacin resistance on MAR index. A total of 254 non-repetitive Gram-negative bacilli recovered from various clinical specimens were used. The isolates were identified and disc susceptibility tests as well as phenotypic detection of extended spectrum β-lactamase were performed on all isolates using standard techniques. MAR index was calculated using a formula. A total of 234 (92.13%) of the isolates had MAR index >0.2. The MAR index of *Pseudomonas aeruginosa* was significantly higher than *Escherichia coli, Proteus mirabilis* and *Raoultella ornithinilytica* ($p<0.05$). The prevalence of ESBL production was higher among those that were ciprofloxacin resistant compared with those that were ciprofloxacin susceptible, with only that among *E. coli* reaching statistical significance ($p=0.0007$). The MAR index of *E. coli* isolates was significantly higher for ESBL-producing and ciprofloxacin resistant strains ($p<0.001$), ESBL-producing but ciprofloxacin susceptible strains ($p<0.05$), and ESBL-non-producing ciprofloxacin resistant strains ($p<0.05$) than for ciprofloxacin susceptible and non-ESBL producing strains. Isolates from the ICU had a significantly ($p<0.05$) higher MAR index than isolates from the Children's Emergency Ward, Labor Ward, Out-patient Department, and Surgical Ward. Antimicrobial agents are misused in the environments where more than 90% of the isolates come from. Only among E. coli strains was a significant correlation between ESBL production and ciprofloxacin resistance seen. Isolates recovered from ICU had a higher mean MAR index. We recommended the implementation of an antibiotic stewardship program.

Keyword: Multiple antibiotic resistance index, β-lactams, Fluoroquinolones, ICU.

Introduction

Unquestionably one of the most important health and economic issues facing our civilization is antimicrobial resistance (AMR) (Kim *et al*., 2023). Antibiotic overuse and abuse have led to the rise of multidrug-resistant bacteria that constitute a severe hazard to people everywhere (Boucher *et al*., 2009; Nordmann and Poirel, 2016; Pham Thanh *et al*., 2016). According to the United Nations Environment Programme, by 2050 AMR will be responsible for almost 10 million deaths every year and if not controlled it will cost around USD 3.4 trillion annually (Soni *et al*., 2023).

In both human and animal healthcare, fluoroquinolones and β-lactam antibiotics are life-saving drugs that are necessary for treating serious infections (De Koster *et al*., 2023). Fluoroquinolones and extended spectrum cephalosporins are the antibiotics of choice in Nigeria for treating infections brought on by a variety of Gram-negative organisms (Ogbolu *et al*., 2011). Prior use of fluoroquinolones and

third generation cephalosporins have been reported as risk factors for extended spectrum β-lactamases (ESBL) production, and the genes that code for ESBL production are usually found on mobile genetic elements that also code for resistance to fluoroquinolones and other antibacterial agents (*Chatterjee et al., 2012; Soraas et al., 2013; Knusden et al., 2014)*. In human therapy, ciprofloxacin is the most widely used antibacterial agent in the world (*Al-Agamy and Zaki, 2012)*. Concomitant fluoroquinolone resistance and β-lactam resistance due to ESBL production results in limited therapeutic options (Kadar and Kumar, 2004; Ramos *et al*., 2016). ESBL-producing isolates have been reported to be more frequent among fluoroquinolone (ciprofloxacin)-resistant isolates (Drago *et al*., 2010; Bonkat *et al*., 2013).

A practical and cost-effective way to identify the source of bacterial resistance is the multiple antibiotic resistance (MAR) index (Woh *et al*., 2023). When assessing health risks, the MAR index is a useful tool for determining if an area has a high or low level of antibiotic use based on the origin of the isolates Davis and Brown (2016). An MAR index value > 0.2 indicates that the isolate is from an environment where antibiotics are abused (Osundiya *et al*., 2013). Antibiotics use in Nigeria is unregulated and over the counter sales of antibiotics without prescriptions are rife (Okeke *et al*., 1999; Omoregie and Eghafona, 2009; Ogbolu, 2013). Against this background this study aims to determine the distribution of MAR index among Gram- negative bacilli and wards/clinics the isolates were recovered from as well as any association between ciprofloxacin resistance and ESBL production.

Materials and Methods

Study Area

The study was cross-sectional and was carried out in the Medical Microbiology Laboratory of the University of Benin Teaching Hospital, Benin City, Nigeria. The hospital is a tertiary hospital with over 860 bed capacity and has a referral status. The hospital serves the need of about 6-10 states in Nigeria and is located in the south-south geopolitical zone in the country. The hospital is located on latitude 6.3903°N and longitude 5.6118°E. Ethical approval for the study was sought and obtained from Edo state Ministry of Health through their letter referenced HA.737/1/3.

Bacterial isolates

A total of 264 Gram- negative bacterial isolates were recovered from clinical specimens sent to the Medical Microbiology Laboratory, University of Benin Teaching Hospital, Benin City, for microbiological analysis between 6th January, 2020 and April 2nd 2020. These isolates were identified using Microbact 24E (Oxoid, England). Information on the specimens, age and gender of patients and wards/clinics the isolates were recovered from was obtained from the laboratory records.

Susceptibility test

Disc susceptibility test was done using the British Society for Antimicrobial Chemotherapy (BSAC) method (Andrews, 2009). Briefly, overnight pure culture of bacterial isolates on nutrient agar was suspended in sterile distilled water and the turbidity matched with 0.5 McFarland standard. Once matched, a sterile cotton-wool swab was dipped in the organism suspension and excess liquid was removed by turning the swab on the side of the test tube. The entire surface of the Mueller-Hinton agar plate (Titan Biotech Limited, India) was seeded by swabbing in three directions with the swab. The following discs amoxicillin-clavulanate (30µg), cefuroxime (30µg), ceftazidime (30µg), piperacillin-tazobactam (30-6µg), imipenem (10µg), meropenem (10µg), amikacin (30µg), ofloxacin (5µg), ciprofloxacin (5µg), levofloxacin (5µg), nitrofurantoin (200µg) and sulfamethoxazole-trimethoprim (23.75-1.25µg) (Oxoid, England) were placed on the seeded agar plate (6 discs/plate). The plates were incubated overnight at 37°C. After overnight incubation, the zone diameter was measured with the aid of a transparent ruler and a standard chart was used to categorize the results as susceptible, intermediate or resistant.

Phenotypic detection of ESBL

A modification of the double disc synergy test described by Nagano *et al*. (2004) was used. Briefly, test organisms were emulsified in sterile

water and the turbidity matched with 0.5 McFarland standards. Once matched, a sterile cotton wool swab was dipped into the organism suspension and excess liquid removed by turning the swab on side of the test tube. The entire surface of Mueller–Hinton agar plate was seeded by swabbing in three directions with the swab. A disc containing 30µg amoxicillin–clavulanate was placed at the centre of the agar plate. Ceftazidime disc (30µg) and cefotaxime disc (30µg) was placed on either side of the amoxicillin–clavulanate at distance of 20mm edge-to-edge. The plates were incubated at 37°C overnight and ESBL production was inferred as positive if there was an expansion of the zone of inhibition between any of the ceftazidime and/or cefotaxime discs and the amoxicillin–clavulanate disc.

Multiple antibiotic resistance (MAR) index

The MAR index was determined as previously described (Lemlem *et al*., 2023). Briefly, MAR index was calculated by dividing the number of antibiotics an organism was resistant to by the total number of antibiotics tested.

$$\text{MAR index} = \frac{\text{number of antibiotics that an isolate was resistant to}}{\text{total number of antibiotics exposed to the isolate}}$$

Statistical analysis

The non-parametric data were analyzed with chi square (X^2) test while the parametric data were analyzed with ANOVA, using the statistical software INSTAT® (Graph Pad Inc., La Jolla, CA, USA).

Results

Out of the 254 clinical isolates used in this study, 92.13% had MAR index >0.2. Box and Whisker plot was done for isolates that are ≥ 3 in number (Fig I). The MAR index of *Pseudomonas aeruginosa* was significantly higher than those of *Escherichia coli, Proteus mirabilis* and *Raoultella ornithinilytica* ($p<0.05$) (Fig I).

In all isolates, the prevalence of ESBL production was higher among those that were ciprofloxacin resistant compared with those that were ciprofloxacin susceptible, with only that among *E. coli* reaching statistical significance ($p=0.0007$) (Table 1).

E. coli, Enterobacter cloacae and *Klebsiella pneumoniae* were the only isolates that had enough number of ESBL producing and non-producing ciprofloxacin resistant and susceptible isolates to permit statistical comparison. Among *E. coli* isolates, strains that were ESBL-producing and ciprofloxacin resistant ($p<0.001$), ESBL-producing but ciprofloxacin susceptible ($p<0.05$) and ESBL-non-producing ciprofloxacin resistant ($p<0.05$), had significantly higher MAR index than strains that were ciprofloxacin susceptible and non-ESBL producing (Fig II).

The MAR index of isolates recovered from ICU were significantly ($p<0.05$) higher that the MAR index of isolates recovered from Children emergency ward, Labour ward, Out-patient Department and Surgical ward (Table 2).

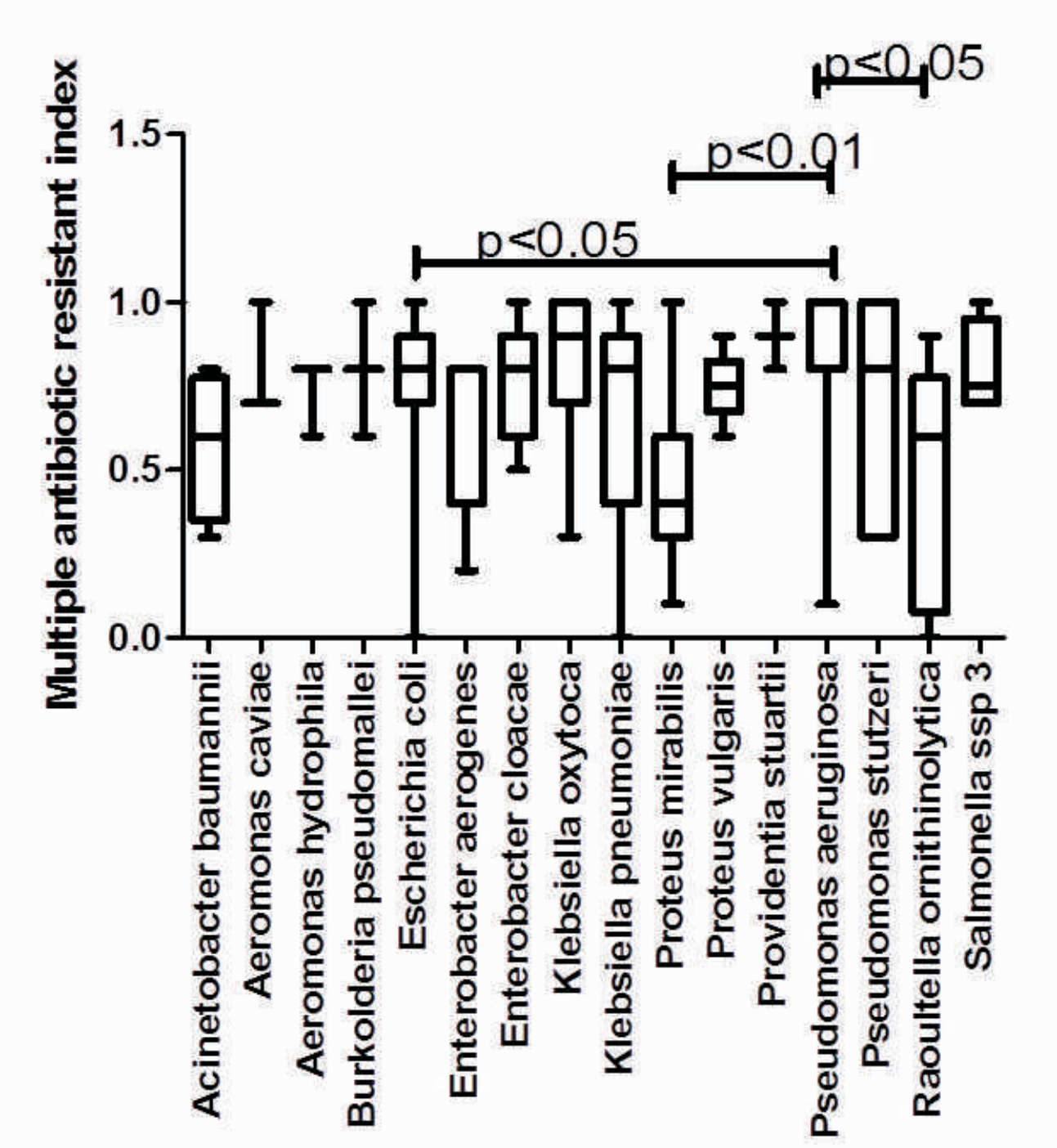

Fig I: Box and Whisker chart of multiple antibiotic resistant index of isolates

Table 1: Association between ciprofloxacin resistance and ESBL production among isolates

Isolates	Ciprofloxacin susceptibility		No, positive for ESBL (%)	P- value
Citrobacter amalonaticus (n =1)	R	1	1 (100.00)	NA
	S			
Citrobacter youngae (n = 2)	R	1	0	NA
	S	1	0	
Cronobacter sakazakii (n=2)	R	2	0	NA
	S			
Enterobacter aerogenes (n=7)	R	5	3 (60.00)	1.0000
	S	2	1 (50.00)	
Enterobacter agglomerans complex (n=2)	R	1	0	NA
	S	1	0	
Enterobacter cloacae (n=11)	R	8	3 (37.50)	0.4909
	S	3	0	NA
Enterobacter hormaechei (n=1)	R	1	1 (100.00)	
	S			
Escherichia coli (n=83)	R	66	43 (65.15)	0.0007
	S	17	3 (17.65)	
Hafnia alvei (n=1)	R	1	0	NA
	S			
Klebsiella oxytoca (n=10)	R	9	8 (88.89)	0.2000
	S	1	0	
Klebsiella pneumoniae (n=27)	R	11	7 (63.64)	1.0000
	S	16	11 (68.75)	
Klebsiella terrigena (n=1)	R	1	1 (100.00)	NA
	S			
Morganella morganii ssp. Morganii (n=1)	R	1	0	NA
	S			
Proteus mirabilis (n=9)	R			
	S	9	4 (44.44)	NA
Proteus vulgaris (n=6)	R	5	1 (20.00)	1.0000
	S	1	0	
Providencia stuartii (n=3)	R	2	1 (50.00)	1.0000
	S	1	0	
Raoultella ornithinolytica (n=8)	R	3	0	NA
	S	5	0	
Salmonella ssp. 3B (n=4)	R	3	1 (33.33)	1.0000
	S	1	0	

Serratia fonticola	R	1	0	1.0000
(n=2)	S	1	1 (100.00)	
Serratia liquefaciens	R			
complex (n=1)	S	1	0	NA
Serratia odorifera	R			
biogp 1 (n=1)	S	1	0	NA
Acinetobacter	R	1	0	NA
baumannii (n=4)	S	3	0	
Acinetobacter	R	1	0	NA
haemolyticus (n=1)	S			
Aeromonas caviae	R	1	0	NA
(n=3)	S	2	0	
Aeromonas hydrophila	R	3	2 (66.67)	NA
(n=3)	S			
Burkholderia	R	2	0	NA
pseudomallei (n=3)	S	1	0	
Pseudomonas	R	35	1 (2.86)	0.5143
aeruginosa (n=50)	S	15	1 (6.67)	
Pseudomonas	R	3	0	NA
stutzeri (n=5)	S	2	0	
Vibrio vulnificus (n=1)	R	1	0	NA
	S			
Yersinia ruckeri (n=1)	R			
	S	1	0	NA

R= resistant; S= susceptible; NA= not applicable

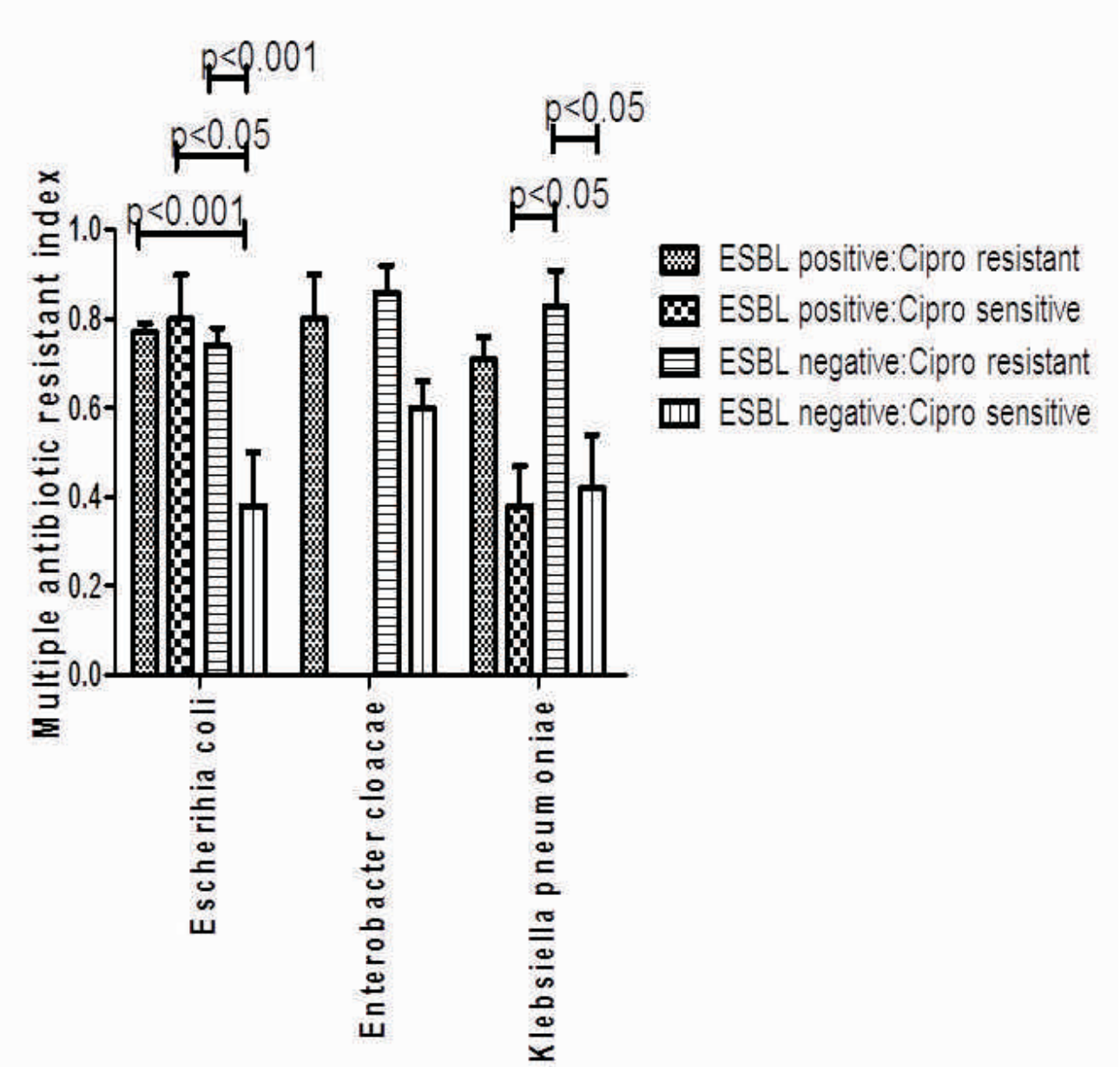

Fig II: Comparison of MAR index of ESBL-producing and non-producing ciprflofoxacin-resistant and –susceptible isolates

Table 2: Distribution of MAR index among isolates from various wards/clinics

Wards/Clinics	No. of isolates	Multiple antibiotic resistance index	
		Range	Mean ± standard deviation
Adult emergency	31	0.3 – 1.0	0.75 ± 0.17
Children emergency	19	0.0 – 1.0	0.67 ± 0.28
Eye ward	1	0.0	NA
Geriatric ward	14	0.0 – 1.0	0.64 ± 0.36
Gynaecology ward	18	0.1 – 1.0	0.75 ± 0.23
Intensive care unit*	11	0.7 – 1.0	0.96 ± 0.09
Labour ward	9	0.1 – 1.0	0.59 ± 0.31
Maternity ward	11	0.0 – 1.0	0.81 ± 0.28
Medical ward	25	0.3 – 1.0	0.81 ± 0.19
Neonatal intensive care unit	6	0.6 – 1.0	0.73 ± 0.16
Neurology ward	7	0.4 – 1.0	0.76 ± 0.27
Oncology ward	1	1.0	NA
Out-patient department	44	0.0 – 1.0	0.69 ± 0.29
Paediatric ward	9	0.2 – 1.0	0.70 ± 0.24
Surgical ward	48	0.0 – 1.0	0.66 ± 0.32

*MAR values from Intensive Care Unit was significantly higher than those from Children Emergency ward ($p<0.05$), Labour Ward ($p<0.05$), Out-patient Departments ($p<0.05$) and Surgical ward ($p<0.01$); NA= Not applicable and not used in analysis

Discussion

The global health crisis of bacterial antibiotic resistance has far-reaching effects (Jain *et al*., 2021). Drug-resistant infections may result in 10 million annual deaths by 2050, with Asia and Africa predicted to account for 90% of these fatalities (Islam *et al*., 2019). The majority of Nigerians only visits the hospital when a disease becomes life-threatening and uses all available quick fixes to lessen the burden of illness, including inappropriate medication use that fuels the rise in antimicrobial resistance (Ichoku and Fonta, 2009). MAR index is an inexpensive tool for tracking bacterial resistance (Woh *et al*., 2023).

The finding that over 90% of isolates had MAR index >0.2 agrees with the fact that antimicrobial usage is unregulated in Nigeria and over the counter sales of antibiotics without prescriptions are rife (Okeke *et al*., 1999; Omoregie and Eghafona, 2009; Ogbolu, 2013). Inappropriate use of antimicrobials in most Nigerian hospitals (Ogunleye *et al*., 2022; Sekoni *et al*., 2022) may account for the high MAR index observed in this study. Empiric therapy is usually based on susceptibility patterns within a given location. The high MAR index observed in this study may necessitate the determination of new susceptibility pattern. Proper implementation of antibiotic stewardship programme is advocated.

Among the various clinical isolates used in this study, the MAR index of *Pseudomonas aeruginosa* was significantly higher than the MAR index of *E. coli* ($p<0.05$), *Proteus mirabilis* ($p<0.01$) and *Raoultella ornithinilytica* ($p<0.05$). Because of its thin outer membrane, which has a permeability of only 1/100 that of the outer membrane of *E. coli*, *P. aeruginosa* displays intrinsic resistance to various antimicrobial agents (β-lactam and penem group of antibiotics) (Bush *et al*., 1995; Martin-Loeches *et al*., 2013). Although some other

mechanisms, such as the efflux system, which expels antibiotics from the bacterial cell, and the production of antibiotic inactivating enzyme, are also to blame for their inherent resistance (Okomoto *et al*., 2001; Paul *et al*., 2004; Poole, 2005; Rello *et al*., 2006; Pachori *et al*., 2019). The highly diverse pathogen that this bacterium is, though, is able to adapt to its environment. The induced response helps bacteria survive and produces antibiotic resistance when they are under antibiotic selective pressure (Pachori *et al*., 2019). This may explain the finding in this study.

The finding that there was an association between ESBL production and ciprofloxacin resistance in *E. coli* agrees with previous reports (Tolun *et al*., 2004; Bonkat *et al*., 2013; Maheshwari *et al*., 2016). ESBL producing isolates frequently carry genes that confer resistance to different classes of antibiotics, including sulfonamides, aminoglycosides, and fluoroquinolones (Rodriguez-Bano *et al*., 2004). In Nigeria, fluoroquinolones and β-lactam antibiotics are among the most widely used (Ogunleye *et al*., 2022; Sekoni *et al*., 2022) and fluoroquinolones (ciprofloxacin) usage is a risk factor for ESBL production (*Chatterjee et al., 2012; Soraas et al., 2013; Knudsen et al., 2014)*. *E. coli* has been reported as the most prevalent Gram-negative bacilli recovered from clinical specimens (Amsalu *et al*., 2017; Thapa *et al*., 2017). This may explain the findings in this study. The non-significant association between ESBL production and ciprofloxacin resistance in other isolates had also been previously reported (Tolun *et al*., 2004).

In this study, the mean MAR index of isolates recovered from Intensive Care Unit (ICU) was higher than the ones from other wards. Ghenea *et al*. (2021) reported that isolates from ICU had the highest resistance. We recently reported the presence of carbapenemase-producing isolates harboring genes for VIM and NDM β-lactamases among patients in ICU (Ibadin *et al*., 2023). The high levels of microbial resistance observed in ICUs are thought to be caused by low immunity, the use of invasive medical devices, and the administration of multiple antibiotics (Ghenea *et al*., 2021; Ibadin *et al*., 2023). To lower the high levels of resistance seen in ICUs, an antibiotic stewardship program should be implemented.

Conclusion

Over 90% of the isolates are from environments were antimicrobial agents are abused. A significant association between ESBL production and ciprofloxacin resistance was observed only among E. coli strains. Mean MAR index was higher among isolates recovered from ICU. Implementation of antibiotic stewardship program is advocated.

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