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Prevalence of Betalactamase Producing and Multidrug Resistance Index of Enterobacteriacea from a Tertiary Hospital in Kaduna Nigeria

Adejo Peter O., Ajibade G. A. and Muhamed Jamiu A.

Abstract:- In the last two decades, increase in multidrug resistance (MDR) of nosocomial bacterial has become a major health challenge worldwide. Enterobacteriaceae are significant causes of serious infections, and many of the clinical important members of this family are becoming increasingly resistant to currently available antibiotics. In this study, one hundred and thirteen non-repeated multidrug resistant enterobacteriaceae were collected from a tertiary hospital for the study and screened for betalactamase production using penicillin-phenol red reagent. *E. coli (38), K. Pneumonae (20), Shigellasp (24)* and *Salmonella sp (31)* were the organisms of interest. Results showed the isolates had a MARI of 0.6 and 48.67% of isolates were betalactamase producers. Isolates were observed to be resistant to various antibiotics with Augmentin having the highest mean resistance rate of 0.98, Ampiclox 0.84, Perfloxacillin 0.82, Erythromycin 0.81, Zinacef 0.79, Amoxicillin and Ofloxacillin 0.77, Septrin and Rocephin 0.75, Chloramphenicol 0.67, Ciprofloxacillin 0.59, Gentamycin 0.56 and streptomycin 0.44. Multi drug resistance in isolates were very high with isolates been resistant to at least six antibiotics and this is linked with the production of betalactamase which render betalactam antibiotics infective. There is need for closer monitoring of drug resistant trends and betalactamase production in both clinical and community settings. This will go a long way in reducing the spread of antibiotic resistance.

I. INTRODUCTION

Antibiotic resistance is the ability of bacteria or other microbes to resist action of antibiotic agents. This happens when an antibiotic no longer inhibits bacterial growth effectively. The microbes are said to be resistant when they survive and continue to multiply in the presence of therapeutic levels, of said antibiotics (Haley, 2015).

In the last two decades, increase in multidrug resistance (MDR) of nosocomial bacterial has become a major health challenge worldwide. Health care professionals are concerned by this development as bacterial infections are becoming resistant to antibiotics of last resort which are highly potent and broad spectrum antibiotic which are administered for the treatment of life threatening infections such as septicemia, tuberculosis, UTI etc.(Rachna*et al*, 2013:Yanga *et al.,2020*).

The recent observed increase in MDR could be attributed to the abuse of medication and general overuse of antibiotics (Nduche*et al*, 2016; Idris*et al*, 2015; Angela *et al*, 2011). Much antibiotic use and misuse is driven by patients, farmers and the general populace who demand antibiotics for real or presumed infections and procure them from unsanctioned source even when they are not prescribed. Resistance is accelerated by selection pressure from inappropriate antimicrobial use while the spread of resistant organisms from one person to another is promoted by poor infection prevention and control along with poor sanitary practices (Rosina *et al*, 2009).

Microbes are diverse and their ability to adapt to various challenges has seen them demonstrate great flexibility- being found in all environments, surviving high pH, temperature and pressure. Their high populations, showing great genomic plasticity, along with the ability for genetic exchange between non-related species, has seen them adapt to resist various antibiotics (Michael and Stuart, 2007). The rise of antibiotic resistance is a global challenge and bacterial antibiotic resistance once developed, is slow or difficult to reverse along with the fact that therapeutic options for the control of such are limited (Pei Wei *et al*., 2015; Angela *et al*, 2013). Poor surveillance and monitoring of trends in the emergence and spread of antibiotic resistance has led to the outbreak of epidemics. This has resulted in the loss of lives, increased cost of treatment and prolonged stays in hospitals. The spread of antibiotic resistance is increased with poor sanitation and preventive measures with an increased risk of nosocomial infections (Yusuf *et al*, 2014). Therapeutics which are available to treat some infections could cause more harm than good either by their side effects or killing off of beneficial organisms in the gut or target site of action. Drug development is slow to address the rapid rise of antibiotic resistance thereby leaving a gap in the demand and supply of therapeutics to manage emerging resistance of infectious agents (Nduche*et al*, 2016).

Hydrolytic inactivation by β–lactamases is the most elusive mechanism to β–lactam antibiotics. Most alarming is the fact that, a single base change in gene encoding β–lactamase can change the substrate specifity of the enzymes as is seen in the various types of enzyme groups identifiable in contemporary times. These include the TEM, SHV etc. (Julian 1994; Sabhghatulla*et al*, 2015; Gianfranco and Gerard, 2010; Michael and Stuart, 2007). Recent studies showed a high incidence of MDR bacterial implicated in nosocomial infections (Chike*et al*, 2017). Nosocomial infections arise from clinical setting and clinical epidemiological reports infection rates of up to 50% of these pathogens. The most common of which are *Staphylococcus aureus*, *Pseudomonas aeriginosa* and *Acinetobacterbaumanmii*. There are also reports of multiple

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drug resistance (Pai Wei *et al*, 2015). Multidrug resistance of *E. coli*to ampicillin and tetracycline, TMP/SMX and fluoroquinolones is at an all-time high. In addition, multidrug-resistant strains that produce extended-spectrum beta-lactamases (ESBLs) have emerged as an important cause of community-acquired UTI and sepsis. ESBLs can hydrolyze most beta-lactams, including penicillins and broad-spectrum cephalosporins and monobactams, but not carbapenems
(imipenem, meropenem, doripenem, ertapenem) (Munita and Arias, 2016).

Other pathogens such as *K. pneumoniae* are also now widely distributed in hospitals and increasing being isolated from community acquired infections (Rosina *et al*, 2009).

A recent report by the Nigerian Federal Ministries of Agriculture, Environment and Health in 2017 on the use and resistance of antimicrobials shows high MDR bacteria cases from various parts of the country. Pathogens highlighted include*; E. coli*, non-thyphiodal and thyphoidal*Salmonella, Shigella species*, *Vibrio cholerea*, and other urinary tract infections. Although it must be noted that there are no accurate data on the extent, pattern and trend of antibiotic resistance in Nigeria, high level of multidrug resistance of up to 100% have been reported (FMAEH, 2017). According to Idris*et al.* (2015), common antibiotic resistant pathogens in Nigeria are; Methicillin resistant *Staphylococcus aureus*(MRSA), Vancomycin-resistant *Staphylococcus aureus* and *Enterococci*, multidrug resistant gram-negative bacteria such as those with extended spectrum β-lactamase(ESBL) resistance and carbapenem-resistant *enterobacterioceae*, multidrug and extensive drug resistant *Mycobacterium tuberculosis*(MDR-TB and XDR-TB). Nkechukwu*et al.* (2014), reported on the *Pseudomonas* species expressing extended spectrum β-lactames (ESBL). Currently marketed β-lactamase inhibitors are not active against all beta-lactamases and reports of resistance to inhibitors are emerging. There is need for new inhibitors of beta-lactamases to checkmate resistance to beta-lactam drugs (Zeighampour*et al*, 2014). To address the need for new therapeutics with novel mode of action against resistant pathogens, plants reportedly having antimicrobial properties are being investigated to ascertain their viability as antimicrobial agents. Identifying plants with activity against enzymes of MDR bacteria which hydrolysis drugs will drive discovery of new drug agents, which will address the need for therapeutic agents against MDR bacteria with lesser side effects or those more effective than currently available antibiotics.

II. METHODOLOGY

Bacteria were collected from the microbiology laboratory of the Nigerian Air force hospital Kaduna. 113 non-repeated strains isolated from stool, urine, skin, nasal and virginal swabs were used in the study. Pure cultures of test isolates were subcultured and stored at 4^0C until required for use. Organisms of interest *were E.coli, K. pneumoniae, Shigella sp.* And *Salmonella sp.*The bacterial isolates were identified based on their cultural and biochemical characteristics using methods described by Cheesbrough 2000.

A. Determination Of Antibiotic Sensitivity And Multiple Antibiotic Resistance Index

Bacteria isolates were tested for their sensitivity towards antibiotics using the Kirby-Buer disk diffusion method. 0.5 McFarland suspension of isolates was seeded unto Mueller Hinton agar plates and sensitivity discs of antibiotics were placed on the seeded plates using sterile forceps. The plates were incubated overnight at 37^0C. After incubation, the zone of inhibition was read to the nearest millimeter and interpretations made according to cut off provided by CLSI. Multidrug resistant isolates were defined as those resistant to three or more classes of antibiotics(carbapenems, penicillin, cephalosporins, monobactams, fluoroquinolones and aminoglycosides) (Raji et al., 2013).

Multiple antibiotic resistance index MAR was determined according to methods described by Ezeador*et al.* (2017) using the formula

MARI=A/B

Where

A =number of antibiotics isolate is resistant to

B = total number of antibiotics tested

MARI >0.3 indicative of the wide use of antibiotics against the isolate.

B. Detection Of Betalactamase Producing Isolates

To test isolates for betalactamases production, 100µl of penicillin-phenol red reagent was dispensed into microtitre wells and test organisms were then suspended in the wells. The wells were observed for colour change. A change in color from purple-pink to yellow within 15 minutes was regarded as positive for betalactamase production and no color change within that time was indicative of no betalactamase production(Bidya and Suman, 2014).

III. RESULTS AND DISCUSSIONS

Figure 1 shows the MARI for the test isolates. *E. coli* has a mean value of 0.54, *Shigell*a 0.59, *Salmonella sp.* 0.68 and *Klebsiella sp.* had a mean value of 0.65. There was no significant difference in the MARI of the test isolates at $p<0.05$ and 95 CI. The tests isolates showed a mean MARI of 0.60.

The resistance of isolates to antibiotics measured as resistance rate is shown in figure 2. Resistance was recorded to be highest against Augmentin(0.98) followed by Ampiclox(0.84) and the least resistance was observed against streptomycin (0.40). Others wereZinacef(0.80), Erythromycin(0.81), Pefloxacillin (0.82), Ofloxacillin (0.78), Amoxicillin (0.78), Septrin (0.75), Gentamycin(0.56), Rocephin (0.75), Chloramphenicol (0.67), and ciprofloxacin (0.60).

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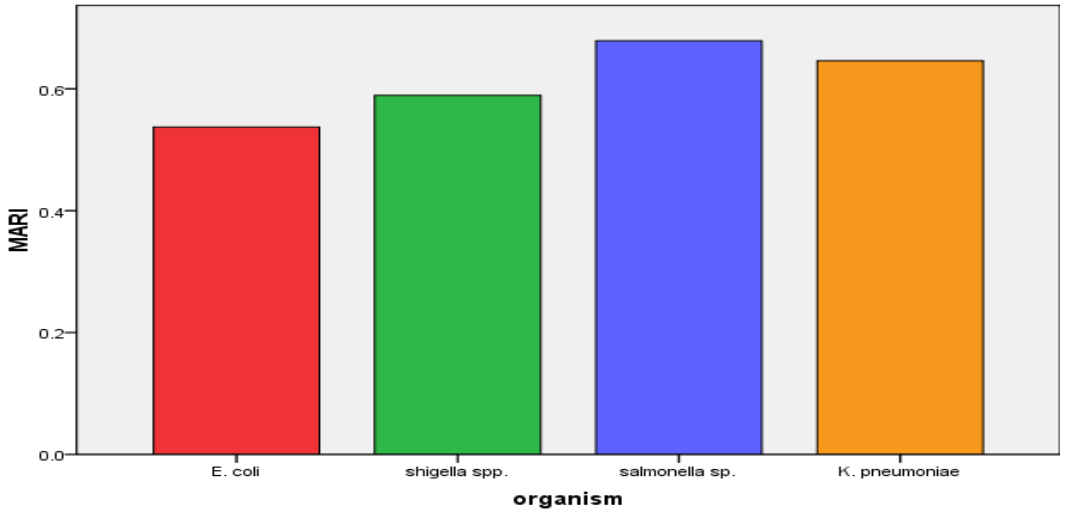

Fig. 1 : Multiple Antibiotic Resistance Index of Test Isolates

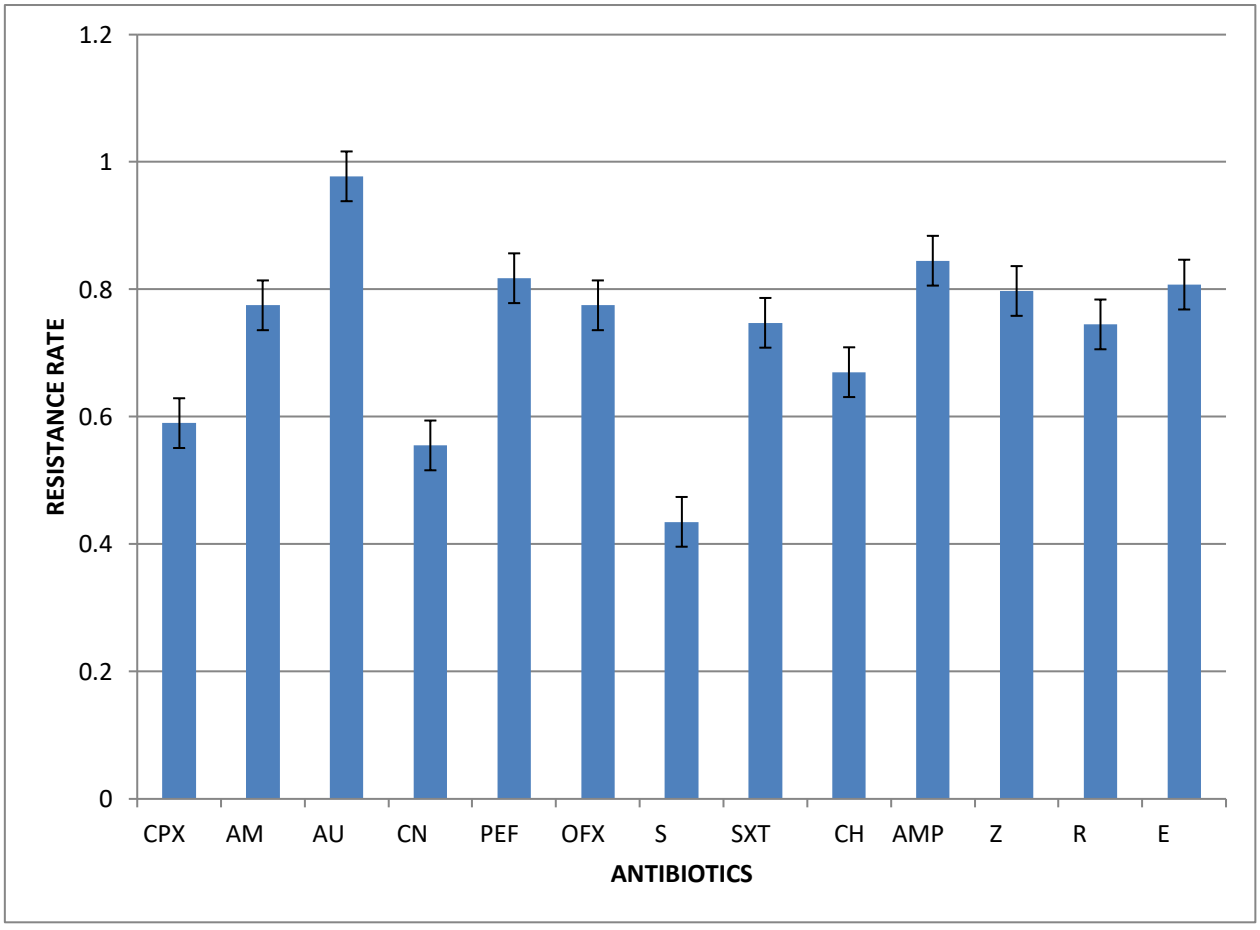

Fig. 2 Resistance Rate of Isolates to Antibiotics

CPX- CIPROFLOXACIN AM - AMOXACILLIN AU- AUGMENTIN CN- GENTAMYCIN PEF- PEFLOXACIN OFX- OFLOXACIN S- STREPTMYCIN SXT- SEPTRIN CH- CHLORAMPHENICOL AMP- AMPICILLIN Z- ZINACEPH R- ROCEPHIN E- ERYTHROMYCIN

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OGARNISM	NO TESTED	BETALACTAMASE PRODUCING ISOLATES	%
E. COLI	38	23	60.52632
SHIGELA SPP.	24	12	50
SAMONELA SPP.	31	12	38.70968
K. PNEUMONEA.	20	8	40
Total	113	55	48.67257

Table 1: Prevalence of Betalactamase Isolates

A total of 113 isolates were screened for batalactamase production. 48.67% were positive for betalactamase production. Among *E.coli* isolates 60.53% were betalactamase producers, 50% in *Shigella* while *Salmonella* and *K. pneumoniae* had 38.7% and 40% betalactamase producing isolates respectively.

The multiple antibiotic resistance index is indicative of wide use of antibiotics against pathogenic bacteria .The isolates showed high level of resistance to the antibiotics tested.. The study revealed a mean MARI of 0.60, which indicates that at least isolates were resistant to six antibiotics tested. In *E. coli* isolates, the mean value is 0.5, *Shigella sp.* 0.59, *Salmonella sp*. 0.68 and *Klebsiellapneumoniae* 0.65 respectively. This is indicative that the isolates have a broad-spectrum resistance in bacteria isolates. This is in concordance with Tope *et al*.,(2016) who reported 63% and 70% of isolates resistant to at least 3 antibiotics and 18% and 41% to at least 10 antibiotics. Similar reports were also given by Raji et al (2013) who reported 51% of study isolates resistant to at least 3 and 28% to at least 5 classes of antibiotics; Kilonzo-Nhagne*et al.,* (2016) who reportd 0.62 MARI value for *E.coli, Pontoea spp*. and 0.8 for *Salmonella spp*. and *K. terrigene* and Ugwu*et al*., (2020) who reported MARI of > 0.2 in 100% of *Klebsiella spp. isolates*, 57% of *E.coli* Isolates and 87% of *Salmonella spp.*

To individual antibiotics, Augmentin had the highest mean resistance rate of 0.98 (Figure 4.2). Ampiclox had 0.84, Perfloxacillin 0.82, Erythromycin 0.81, Zinacef 0.79, Amoxicillin and Ofloxacillin 0.77, Septrin and Rocephin 0.75, Chloramphenicol 0.67, Ciprofloxacillin 0.59, Gentamycin 0.56 and streptomycin 0.44. This is in agreement with Magwenzi*et al*(2017), who reported 65% resistance to gentamicin, 100% reistance to ampicillin, 82% to ciprofloxacin, 53% to chloramphenicol and 1% to ertapenem in positive ESBL producing enterobacteriaceae. It was also demonstrated that 44.2% of the test isolates were susceptible to Gentamycin, 43.75% to Streptomycin, 26.79% to Chloramphenicol, 32.74% to Ciprofloxacin, 22.12% to Septrin, 19.475 to Perfloxacillin, 2.35% to Ampliclox, 15.29% to Zinacef, 1.16 to Erythromycin, 17.70% to Amoxicillin and 4.42% to Augmentin. High resistance rate of *enterobacteriaceae* isolates observed in this study could be attributed to the indiscriminate abuse of antibiotics. Thesepathogens, most often are localized in the gastrointestinal tract and have a high chance for genetic exchange. This exchange of genetic material pass on resistant gene in plasmids or other genetic elements from an antibiotic resistant organisms to susceptible organisms. The accumulation and subsequent transfer of resistant genes between organisms is invariably responsible for the high MARI and resistant rates observed in this study. It must be also be noted that high resistance rate and MARI observed, could be linked to co-resistance arising from the expressing of a gene to a particular antibiotic leading to activation of resistance of another antibiotic. For instance, as reported by Williemsem*et al*. (2009) where treatment with gentamicin leads to selection of ESBL gene carrying organisms because, the two resistant genes are linked or carried on the same genetic element (i.e. plasmid). Extended resistance observed in this study may also be attributed to increased expression of multidrug efflux pump or reduced porin production. Some betalactam antibiotics are broad spectrum antibiotics and therefore, their importance in clinical use. They are the most prescribed antibiotic. In this study, a high resistance to betalactam antibiotics was observed (see Figure 4.2) along with a significant betalactamase production in the test isolates. The betalactamase prevalence coupled with high level of antibiotic resistances observed in this study infer drug failure in treatment of infections which might arise from *enterobacteriaceae* which harbor these resistance elements in their genome. This spells trouble in a case of an outbreak in the community as therapeutic success might not be achieved.

The study showed, 48.67% of test isolates were positive for betalactamase production (Table 1). The result showed that 60.53% *of E. coli* isolates were betalactamase producers, 50% of *Shigellasp.* 38.7% of *Salmonella sp.* and 40% of *K.pneumoniae* were positive for betalactamase production. This high prevalence could be attributed to high demand and use of betalactam antibiotics for treatment of enterobacteriaaceae infections. This finding is in agreement with Giwa*et al.,* (2018) who reported 50% and 40% producers in *E.coli and K. pneumoniae*isolates respectively.

IV. CONCLUSION

Multidrug resistance is a major concern as therapeutic interventions for infections of pathogenic organisms especially bacteriaceaes are failing. Findings from this work underscores the facts that multidrug resistance could be linked to the production of betalactamase by pathogenic organisms. This calls for pharmaco-vigilance and a multi facet approach to cub the development and spread of multidrug resistance of enterobateriaceas.

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