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ORIGINAL ARTICLE

Multiple Antibiotic Resistance Index of *Klebsiella pneumoniae* Isolated from Clinical Specimens in a Tertiary Hospital in Benin City, Nigeria

Ogefere HO and Idoko MO*

Department of Medical Laboratory Science, University of Benin, P.M.B. 1154, Benin City, Nigeria.

ABSTRACT

Background: *Klebsiella pneumoniae* is a commensal of the gastrointestinal tract but has been associated with hospital associated- and community infections. In this investigation, *K. pneumoniae* that was isolated from several clinical specimens of patients at a tertiary hospital in Benin City was evaluated for its multiple antibiotic resistance (MAR) index.

Methods: A total of 100 clinical isolates of *K. pneumoniae* from the hospital's inpatients and outpatients were employed. By using conventional microbiological techniques, isolates were identified. Antibiotic susceptibility testing was carried out and MAR index was calculated.

Results: The susceptibility test showed that *K. pneumoniae* exhibited high level of multidrug resistance with a total of 24 isolates resistant to the 12 antibiotics tested. The MAR index ranged from 0.42 to 1.00 with the mean value of 0.7994. The *K. pneumoniae* isolates from clinical specimens of inpatients showed a higher MAR index of 0.82 than that of outpatients (0.74).

Conclusion: These findings present a major public health concern. Concerted efforts that emphasize antimicrobial stewardship must be put in place to mitigate this problem.

Keywords: Antimicrobial drug resistance; *Klebsiella pneumonia*; Tertiary hospitals; Nigeria.

INTRODUCTION

Antimicrobial resistance (AMR) is a widespread issue, particularly in developing nations; it progressively jeopardizes the outcome of numerous ailments that were curable until recently and are nevertheless prevalent in Africa. Public health is also at risk from AMR in both wealthy and developing nations. Treatment for infectious diseases has thus become challenging due to bacterial resistance to several medications. Antibiotic-resistant bacteria infections, particularly those caused by multidrug-resistant (MDR) strains, can result in major health issues such as extended hospital stays, unsuccessful treatments, and even death.[1] The indiscriminate use of antimicrobial drugs in agriculture, aquaculture, and human and animal medicine is one of the main causes of bacterial resistance.[2] Multiple AMR is a common result of the presence of plasmids containing one or more resistant genes, each of which encoding a single phenotype. These genes can be transferred to other bacteria of the same species or different ones. Bacteria develop resistance to antibiotics through a variety of mechanisms, some of which are dominant and identifiable.[1] An efficient, reliable, and reasonnably priced method for determining the source of antibiotic-resistant bacteria is the multiple anti-biotic resistance (MAR) index. MAR index is calculated as the ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics the organism is exposed to.[2] If the MAR index is greater than 0.2, the isolate originates from a source that uses antibiotics often and/or heavily.[3]

Klebsiella pneumoniae is a Gram-negative, rod shaped, non-motile, facultative anaerobic bacteria. *Klebsiella pneumoniae* is an emerging threat to human health that causes both endemic and epidemic infections. Most hospital-acquired infections caused by *Klebsiella pneumoniae* cause a challenge in their treatment because of the development of MDR strains. The resistance of *Klebsiella pneumoniae* strains to different generations of cephalosporins, especially the third generation, was first reported in 1981; since then, these bacteria have become more resistant to several antibiotics[1]. This may

Corresponding author:
Email: idoko_meshach@yahoo.com
Phone: +2348169968873

be due to poor healthcare infrastructure and inappropriate antibiotic use.

Some studies have highlighted the escalating antibiotic resistance in *Klebsiella pneumoniae* strains isolated from clinical specimens in Nigeria.[1,4] Many routinely used antibiotics, including β-lactams (including extended-spectrum cephalosporins and carbapenems),[5,6] fluoroquinolones,[7] and aminoglycosides,[8] have seen a significant rise in resistance. The study of the multiple antibiotic resistance index of *Klebsiella pneumoniae* is of paramount importance given the increasing threat of antibiotic-resistant infections. Research in this field can help define the scope of the issue, direct treatment plans, and influence public health regulations meant to stop the spread of antibiotic resistance.

Not many research works have been documented on the MAR index of *Klebsiella pneumoniae* in Nigeria. Hence there is the need for more research to be done in this area. The aim of this study is to ascertain the multiple antibiotic resistance (MAR) index of *Klebsiella pneumoniae* isolated from clinical specimens in University of Benin Teaching Hospital, Benin City, Edo State, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted at the University of Benin Teaching Hospital (UBTH), Benin City Edo State. The hospital is one of the foremost tertiary hospitals in the South-South geopolitical zone of the country, being 850-bedded and comprising 30 wards. Ethical approval was obtained from the Ethics and Research Committee of the UBTH with reference number: ADM/E22/A/VOL.VII /VII/1483011842.

Source of Bacterial Isolates

One hundred (100) clinical isolates of *Klebsiella pneumoniae* were isolated from inpatients' and outpatients' clinical specimens submitted for routine culture and susceptibility testing at the Medical Microbiology Laboratory, University of Benin Teaching Hospital (UBTH), Benin City. All isolates were identified using conventional methods such as Gram stain, motility testing, and biochemical reactions.[9]

Antimicrobial Susceptibility Test

An antibiotic susceptibility test was performed using the disc diffusion (Kirby-Bauer) method on Mueller Hinton Agar plates. Bacterial suspension was prepared in 0.5 McFarland turbidity standard for each isolate and was swabbed on already prepared Mueller Hinton agar plates. The plates were impregnated with 12 antibiotics discs (using 2 plates comprising 6 antimicrobial discs each), after which were incubated at 37^{o}C for 24hours. The antibiotics used includes; Amoxicillin Clavulanate (30µg), Cefotaxime (25µg), Imipenem/Cilastatin (10/10µg), Ofloxacin (5µg), Gentamycin (10µg), Nalidixic acid (30µg), Nitrofurantoin (300µg), Cefuroxime (30µg), Ceftriaxone Sulbactam (45µg), Ampiclox (10µg), Cefexime (5µg), and Levofloxacin (5µg) (Oxoid, UK). As per the advice of the European Committee on Antimicrobial Susceptibility Testing,[10] zones of inhibition diameter were measured and classified as susceptible, intermediate, and resistant.

Determination of MAR Index

Determination of MAR index was carried out as described by Khan *et al.*[2] In which the number of antibiotics an isolate is resistant to (a) is divided by the total number of the antibiotics used in the study (b). The calculating formula is:

MAR index = a/b where a = number of antibiotics an isolate is resistant to and b= total number of the antibiotics used in the study.

Statistical Analysis

The data from this study were presented and analyzed using SPSS version 21.0 (IBM Inc., USA). It was used to compare means, analysis of variance (ANOVA), and the findings were displayed in bar charts and tables.

RESULTS

The antibiotic susceptibility profile of *Klebsiella pneumoniae* were classified as "resistant", "intermediate" and "susceptible." *Klebsiella pneumoniae* exhibited high levels of resistance to certain antibiotics. Amoxicillin-clavulanate (AUG) showed the highest resistance rate at 97%, followed closely by nalidixic acid (NA) at 95%, cefexime (ZEM) at 95%, ampiclox (ACX) at 90% cefotaxime (CTX) at 89%, nitrofurantoin (NF) at 85%, imipenem (IMP) at 65%, Ceftriaxone (CRO) at 70% and Gentamicin (GN) exhibited a resistance rate of 61%. Levofloxacin (LBC) demonstrated a comparatively lower resistance rate of 54% (Figure 1).

Data on the multiple antibiotic resistance (MAR) index of isolates of *Klebsiella pneumoniae* are shown in Table 1, with results from male and female subjects compared. The range of MAR index values for isolates obtained from female and male individuals was consistent, ranging from 0.42 (minimum) to 1.00 (maximum). Comparing MAR index for isolates from males and females was statistically not significant (p=0.835).

Two main age groups for the pediatric and adult population are presented in table 2 (<18 years and ≥18 years). The MAR index ranges from 0.42 to 0.92, and for the "≥18 years" group, there were 77 isolates with mean MAR index value of 0.82. Comparison of MAR index among

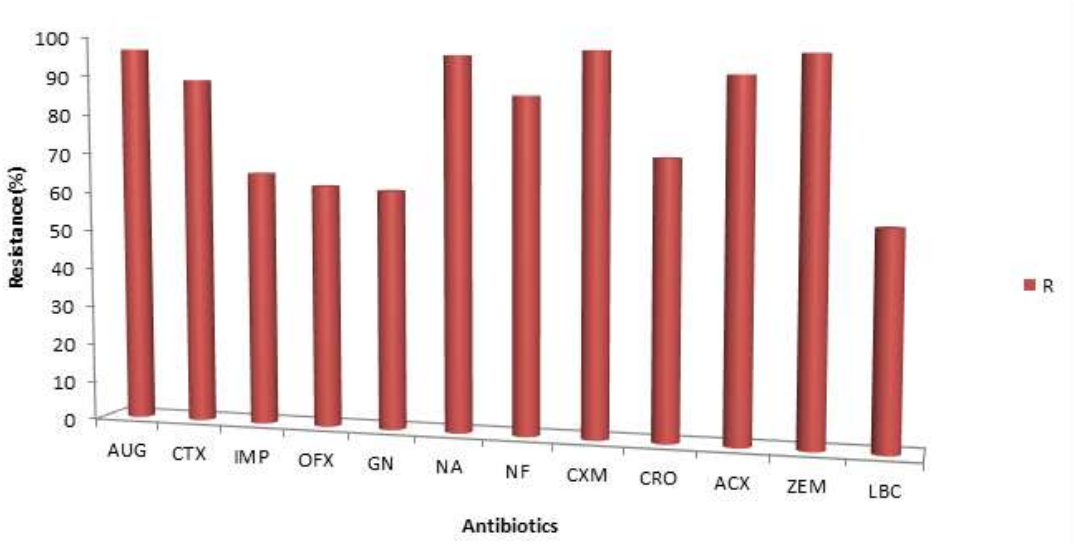

Figure 1: Percentage of *Klebsiella pneumoniae* resistant to different antibiotics used in this study

AUG – Amoxicilin clavulanate; CTX – Cefotaxime; IMP – Imipenem; OFX - Ofloxacin; GN-Gentamycin; NA- Nalidixic acid; NF – Nitrofurantoin; CXM – Cefuroxime; CRO – Ceftriaxone sulbactam; ACX – Amplicox; ZEM – Cefexime; LBC – Levofloxacin; R - Resistant

Table 1: Comparison of the MAR index of *Klebsiella pneumoniae* isolated from male and female patients

Sex	Number of Isolates N=100	Range		MAR Index Mean±SD	P value
		Minimum	Maximum		
Female	57	0.42	1.00	0.79±0.12	0.835
Male	43	0.42	1.00	0.80±0.15	

N – Number tested; MAR Index– Multiple Antibiotic Resistance Index; SD- Standard Deviation

Table 2: Comparison of the MAR index of *Klebsiella pneumoniae* isolated from different age groups

Sex	Number of Isolates N=100	Range		MAR Index Mean±SD	P value
		Minimum	Maximum		
<18	23	0.42	0.92	0.74±0.13	0.028
≥18	77	0.42	1.00	0.82±0.14	

N – Number tested; MAR Index– Multiple Antibiotic Resistance Index; SD- Standard Deviation

Klebsiella pneumoniae isolates from different age groups of patients was statistically significant as the pediatric population had comparatively lower MAR index of *K. pneumoniae* (p=0.028).

Table 3 presents the diverse range of specimens from which *Klebsiella pneumoniae* was isolated from. Among the isolation sources, urine samples were the most prevalent, constituting 27% of the dataset. Other frequently sampled sources included wound swabs (20%), high vaginal swabs (HVS, 6%), and ear swabs (5%). The range of the MAR index values for each specimen is indicated with their mean values. *K. pneumoniae* from ascitic fluid had the lowest MAR index (0.50) while peritoneal fluid and pus showed pan-drug resistance to all antibacterial drugs (MAR index = 1). The distribution of MAR index in relation to source of patients; admitted patients (inpatients) and patients visiting outpatient clinics (outpatients) is shown in table 4. Both groups had the same minimum and maximum value of

MAR index which is 0.42 and 1.00 respectively. Comparison of the mean MAR index and standard deviation for the inpatients (0.82±0.13) and that of the outpatients (0.74±0.15) was statistically significant (p=0.014).

Table 3: Comparison of the MAR index of *Klebsiella pneumoniae* isolated from various clinical specimens

Specimen	Number of Isolates N=100	MAR Index Mean
Abdominal Aspirate	1	0.83
Anal Abscess	1	0.67
Ascitic fluid	1	0.50
Bile	1	0.67
Blood culture	2	1.00
Catheter tip	10	0.92
Ear swab	5	0.92
Endocervical swab	4	0.83
Eye swab	1	0.75
High vaginal swab	6	0.92
Keloid aspirate	1	0.75
Left ear swab	1	0.83
Penile discharge	1	0.92
Peritoneal abscess	1	1.00
Pus (abscess)	1	1.00
Right ear swab	1	0.75
Scrotal abscess	1	0.67
Sputum	3	0.83
Stool	1	0.83
Throat swab	5	0.83
Tongue swab	1	0.67
Umbilical vein catheter	1	0.58
Urethral catheter tip	1	0.92
Urine	27	1.00
Ventriculoperitoneal shunt catheter	1	0.67
Wound aspirate	1	0.83
Wound swab	20	1.00

N – Number tested; MAR Index– Multiple Antibiotic Resistance Index; SD- Standard Deviation

Table 4: Comparison of the MAR index of *Klebsiella pneumoniae* isolated from inpatients and outpatients

Category	Number of Isolates N=100	Range		MAR Index Mean ±SD	P value
		Minimum	Maximum		
Inpatients	72	0.42	1.00	0.82±0.13	0.014
Outpatients	28	0.42	1.00	0.74±0.15	

N – Number tested; MAR Index– Multiple Antibiotic Resistance Index; SD- Standard Deviation

DISCUSSION

Klebsiella pneumoniae is an infamous member of the ESKAPEE pathogens comprising *Enterococus faecium, Staphylococcus aureus. K. pneumoniae, Acinetobacter baumannii, P. aeruginosa, Enterobacter cloacae* and *Escherichia coli*, so named due to their ability to "eskape" and cause hospital associated infection (HAI) in the clinical setting and their genetic pliability that makes them acquire extra chromosomal genetic elements which harbor drug resistance determinants making them MDR. In the study area, *K. pneumoniae* has been associated with outbreak of neonatal sepsis, and several MDR genes have been detected from isolates causing clinical infections.[11,12,13] There's therefore need to determine the MAR index of these isolates so as to evaluate their resistance pattern and highlight its public health significance.

The findings in this study revealed the high resistance rates of *Klebsiella pneumoniae* to commonly used antibiotics such as Amoxicillin-clavulanate, Nalidixic acid, Cefotaxime, Nitrofurantoin and Imipenem. These values were slightly lower than that obtained in Oyo state by Ayandele *et al.*,[1] which had resistance rate of cefotaxime at 93.75%, ofloxacin at 80%, gentamicin at 70% and nitrofurantoin at 89%. This could be due to the difference in strains and geographical location.[14] *Klebsiella pneumoniae* showed sensitivity to Levofloxacin at 42%, followed by Ofloxacin at 31% and Imipenem at 28%. Their sensitivities to these drugs are low compared with another study carried out in Lagos state by Osundiya *et al.*,[4] which had sensitivity to ofloxacin at 91% and imipenem at 95%. The chromosomal mutation at the quinolone resistance determining region (QRDR) seen in *Klebsiella pneumoniae* over the years[7] have made *Klebsiella pneumoniae* resistant to fluoroquinolones and also the acquiring of the blaKPC gene making *Klebsiella pneumonaie* resistant to carbapenems.[5]

From this research work, *Klebsiella pneumoniae* is resistant to more than three classes of antibiotics used in this study. It showed resistance against the β-lactam/β-lactam inhibitor drugs and β-lactam drugs (Amoxicilin clavulanate, cefotaxime, imipenem, cefuroxime, ceftriaxone sulbactam, ampiclox, and cefexime), quinolones (ofloxacin, nalidixic acid, and levofloxacin), aminoglycosides (gentamicin) and the only nitrofuran derivative (nitrofurantoin) which indicates *Klebsiella pneumoniae* as a multidrug resistant organism according to Lee *et al.*,[15] This organism has been extensively studied due to its ability to cause HAIs as well as its ability to acquire plasmids and transposons harboring genes that mediate AMR. In the study area, extended spectrum beta-lactamase (ESBL) enzymes and carbapenemase genes have been detected from *K. pneumoniae* causing clinical infections in UBTH.[11,12] These enzymes mediate resistance to third generation cephalosporins and carbapenem drugs respectively. Similarly, various plasmid types implicated in AMR such as IncF plasmids have also been shown using whole genome sequencing.[11]

Unsurprisingly, there was no significant difference in the MAR index of *Klebsiella pneumoniae* isolates obtained in males and females. This may imply that the rate of exposure to this antibiotic is both the same, being very high. This agrees with several Nigerian studies that have highlighted indiscriminate use of antimicrobials without gender bias that has exerted selective pressure, leading to the proliferation of MDR strains.[11,12] It is also in tandem with a study carried out in Nepal by Jones *et al.*,[16] Also, in this study, the rate of resistance in the pediatric population was lower than the adult counterpart. This agrees with the study carried out by the American Chemical Society.[17] This could be because children and adolescents are generally less exposed to antibiotics than adults, which means they have less opportunity to develop bacterial resistance. Having more isolates from inpatients than from outpatients in this study agrees with the fact that *Klebsiella pneumoniae* causes HAI as well as a community acquired infection, but is more isolated in the hospital environment. This finding agrees with the study carried out in China by Lee *et al.*,[15] The difference in antibiotic resistance rates between inpatients and outpatients could be due to several factors. One possible explanation is that patients who are hospitalized are more likely to have underlying health conditions that require more extensive antibiotic treatment, which can lead to increased resistance. Additionally, hospital environments are more conducive to the spread of antibiotic-resistant bacteria than community settings.[18]

The isolates were collected from various anatomical sites, suggesting that *Klebsiella pneumoniae* has the potential to cause infections in different body regions.[19] Among the isolation sources, urine samples were the most prevalent, constituting 27% of the dataset. This observation aligns with the well-documented ability of *Klebsiella pneumoniae* to cause urinary tract infections.[19] These findings emphasize the significance of tracking and treating *Klebsiella pneumoniae* infections at various body locations. The mean MAR index for the entire dataset was found to be 0.7994. This finding is similar to the 0.8 obtained in Bauchi state by Umar *et al.*,[20] This value suggests that, on average, the isolates exhibited resistance to approximately 80% of the antibiotics tested. This is of great concern and underscores the potential challenge in treating *Klebsiella pneumoniae* infections due to its substantial resistance to commonly used antibiotics. Notably, the dataset's antibiotic resistance profile highlights the urgent need for judicious antibiotic use and the development of alternative treatment strategies.

Conclusion: *Klebsiella pneumoniae* was isolated from various clinical specimens and developed high resistance rate to the four classes of antibiotics used in this study namely β-lactams, aminoglycosides, quinolones and nitrofuran derivatives with high MAR index. This is a serious concern to public health and concerted efforts that prioritize antimicrobial stewardship must be put in place to mitigate this problem.

Authors' contributions: IMO contributed to data collection, laboratory analysis, presentation of result and drafted the manuscript; OHO contributed to the conception, design and interpretation of data. All authors read and approved the final version of the manuscript.

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