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Detection of Quinolone Resistance Genes and Multiple Antibiotic Resistance (MAR) Index of *Klebsiella pneumoniae* isolated from urine in Sokoto, Nigeria

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ABSTRACT:

Introduction: Globally, antimicrobial resistance is a foremost public health challenge, particularly in developing countries. Multiple antibiotic resistance (MAR) index is helpful in analyzing health risk, as well as to check the extent of antibiotic resistance.

Methodology: About four hundred (400) clinical isolates of members of the of the family Enterobacterales were isolated from urine specimens of inpatients and outpatients submitted for routine culture and susceptibility testing at the Medical Microbiology Laboratory, Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. Isolates were identified using colonial morphology and standard biochemical test. Antimicrobial susceptibility testing was conducted using modified Kirby bauer disc diffusion method. The quinolone resistance genes ware detected using Polymerase Chain Reaction.

Result: The isolates were recovered from adult patients with age range between 18-75 years. Majority of the patients were females (60%). The prevalence of *Klebsiella pneumoniae* was 8.75%. and the antibiotic susceptibility profile of *Klebsiella pneumoniae* were classified as either “resistant”, “intermediate” or “susceptible.” It exhibited high levels of resistance to certain antibiotics. Ampicillin showed the highest resistance rate at 26(74.3%), followed closely by Amoxicillin-clavulanate and Ceftriaxone 24(68.6%), Ciprofloxacin 22(62.9%), Gentamicin 20(57.1%), Ceftazidime 19(54.3%), and Levofloxacin 20(55.1%). Amikacin appeared to be the most sensitive drug at 21(60%); then followed by Meropenem at 19(54.3%). The MAR of the isolate ranges from 0.29 for Meropenem to 0.74 for Penicillin G. quinolone resistance genes were detected in 4(11.4%) of the Ciprofloxacin resistance isolates.

Conclusion: The irrational use of antimicrobials is certainly a complex and multifactorial problem in developing countries, and a proper understanding of the problem is necessary for effective control policies.

KEYWORDS: Klebsiella pneumoniae, multiple antimicrobial index, antibiotics, antimicrobial resistance, medical microbiology.

INTRODUCTION

The tribe *Klebsiellae,* which is a part of the Enterobacterales family, includes the genus *Klebsiella*. The organisms bear the name of the German microbiologist Edwin Klebs, who lived in the 19th century. When *Klebsiella spp*. are stained with a Gram stain, they appear pink (negative bacteria) with a noticeable polysaccharide capsule. They are non-motile, straight rod-shaped, and measure 0.3-1μm in diameter and 0.6-6μm in length. The entire cell surface is encased in this capsule, which offers defense against numerous host defensive mechanisms.(1) As an opportunistic bacterium that causes nosocomial infections, *Klebsiella pneumoniae* is the most significant species in the genus in terms of clinical relevance, high death rates in the absence of therapy, including wound contamination and urinary tract infections (UTIs). After *Escherichia coli*, it is the second most frequent cause of UTI, frequently brought on by the use of indwelling catheters.(2) The treatment choices for associated diseases such as pneumonia, liver abscess, meningitis, bloodstream infections, and UTIs are limited due to this clinically significant pathogen's propensity to develop multidrug resistance (MDR).(3)

Globally, antimicrobial resistance is a foremost public health challenge, particularly in developing countries. Misuse of antimicrobials is aided, in developing countries, by their availability over the counter, without a prescription, and through unregulated supply chains.(4) Antibiotic resistance in European healthcare facilities led to high death rates of up to 25,000 individuals annually, costing the continent's healthcare systems some €1.5 billion annually which is N2.62trillion.(5)(6) According to data from the United States Centers for Disease Control and Prevention (CDC), over 20,000 people die each year from infectious diseases that affect over two million people that are resistant to antimicrobial agents.

Multiple antibiotic resistance (MAR) index is helpful in analyzing health risk, as well as to check the extent of antibiotic resistance. The index analysis has been used to differentiate isolates from different sources using antibiotics that are commonly used in the treatment of infectious diseases. (7) The method is less expensive, quicker, simpler to use, and doesn't require costly equipment or specialized training than other techniques of tracking the source of bacteria. When compared to the recognized molecular diagnostic techniques, MAR indexing has been shown to be a more straightforward and affordable way to locate isolation sources.(8) A MAR index value greater than 0.2 indicates high risk source of contamination where antibiotics are often used inappropriately.(9) This study aimed to determine the multiple antimicrobial resistance index of *Klebsiella pneumoniae* isolated from urine in Sokoto state, Northwestern Nigeria.

METHODOLOGY

Study Area

The study was conducted at the Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Northwestern Nigeria. The 850-bedded hospital is one of the foremost tertiary hospitals in the northwest geopolitical zone providing general and specialized patient care to the numerous indigenes of Sokoto state and neighbouring states, and nearby towns and villages of Niger Republic.

Source of Bacterial Isolates

The isolates were obtained from patient samples attending Usmanu Danfodiyo University Teaching Hospital Sokoto. The size, shape, and nature of the isolates, and also their ability to ferment lactose on MacConkey or Cyteine Lactose Electrolyte Deficient Agar were tested. All isolates were first sub-cultured on nutrient agar plates and then incubated for 24 hours at 35 °C. Isolates were further identified using standard biochemical tests which include: Triple iron sugar agar (TSI), citrate, urease and indole.(10)

Antimicrobial Susceptibility Testing

Antibiotic susceptibility test (AST) 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 five to six antibiotic discs, after which the discs were incubated at 37ºC for 24 hours. The antibiotics used included; Amikacin (30ug), Amoxicillin Clavulanate (30µg), Ceftazidime (25µg), Meropenem (10µg), Gentamicin (10µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Ampicillin (10ug) and Levofloxacin (5µg). The AST result was interpreted using Clinical and Laboratory Standard Institute (CLSI 2023) and the zones of inhibition diameter were measured and classified as either susceptible, intermediate, or resistant.(11)

Determination of MAR Index

Determination of MAR index was carried out as described by Krumperman, (1983).(12) 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.

Polymerase Chain Reaction

The quinolone resistance gene (QNR) was detected using Polymerase Chain Reaction and it was conducted as described by Cattoir *et al*., 2007.(13)

Statistical Analysis

Data were analyzed using IBM Statistical Package for Social Science (SPSS) version 25.0. Quantitative variables were summarized using the mean and standard deviation and categorical variables were summarized using frequencies and percentages. The results were presented in the form of tables, charts, and photomicrographs. The level of significance (a) was set at 0.05.

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Table 1: Primers Used For The Study

Gene Target	Primer Name	Sequence (5′ → 3′)	Amplicon Size (bp)	Reference
Universal QNR	QNR-F	GGGTATGGATATTATTGATAAAG	~516	Cattoir *et al*., 2007 (13).
	QNR-R	CTAATCCGGCAGCACTATTA		

RESULTS

About four hundred (400) clinical isolates of members of the of the family Enterobacterales were isolated from urine specimens of inpatients and outpatients submitted for routine culture and susceptibility testing at the Medical Microbiology Laboratory, Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. The isolates were recovered from adult patients with age range between 18-75 years. Majority of the patients were females (60%). The prevalence of *Klebsiella pneumoniae* was 35 (8.75%). and the antibiotic susceptibility profile of *Klebsiella pneumoniae* were classified as either “resistant”, “intermediate” or “susceptible.” It exhibited high levels of resistance to certain antibiotics. Ampicillin showed the highest resistance rate at 26(74.3%), followed closely by Amoxicillin-clavulanate and Ceftriaxone 24(68.6%), Ciprofloxacin 22(62.9%), Gentamicin 20(57.1%), Ceftazidime 19(54.3%), and Levofloxacin 20(55.1%). Amikacin appeared to be the most sensitive drug at 21(60%); then followed by Meropenem at 19(54.3%). (Figure 1).

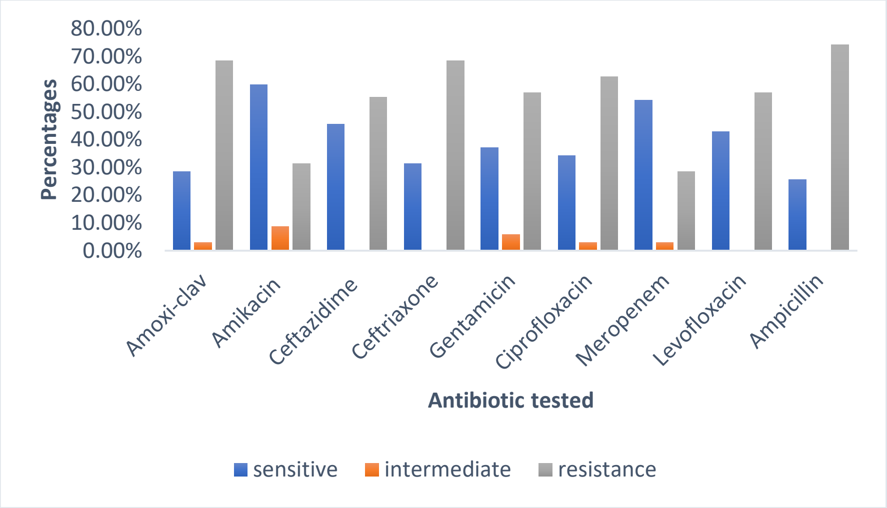

Figure 1: Antimicrobial susceptibility pattern of the isolates

The MAR of the isolate ranges from 0.29 for Meropenem to 0.74 for Penicillin G. The data for the multiple antimicrobial indices are shown below.

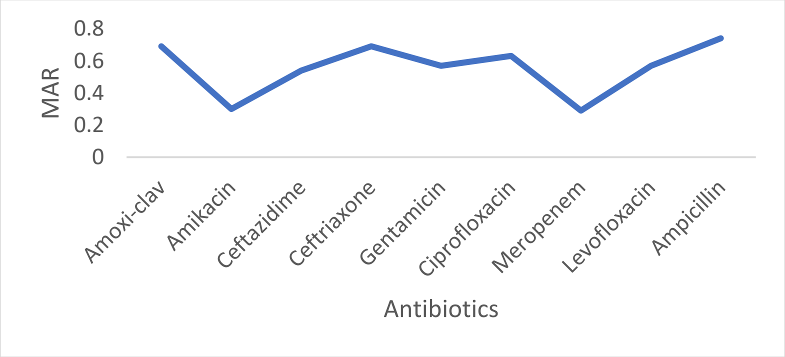

Figure 2: Multiple Antimicrobial Resistance Index of the Isolates against the tested antibiotics

Result of the Polymerase Chain Reaction showed that the QNR was detected in four (11.4%) of the isolates.

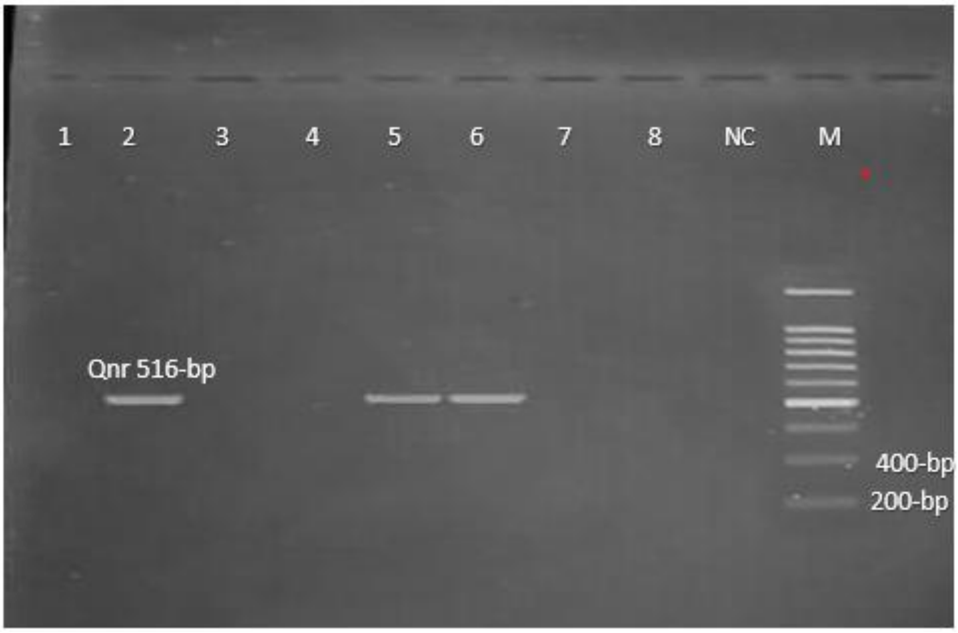

Figure 3: Gel Electrophoresis showing QNR genes

DISCUSSION

The results of this study on the antimicrobial susceptibility have indicated high resistance rate to the commonly used antibiotics in Nigeria and therefore limit therapeutic option. Ampicillin showed the highest resistance rate at 74.3%, followed closely by Amoxicillin-clavulanic acid and Ceftriaxone at 68.6%. Ciprofloxacin at 62.9%, Gentamicin at 57.1% and Ceftazidime at 54.3% and Levofloxacin at 55.1%. These findings are not different from what was reported from other parts of the country, Africa and globally.(14)(15)(16)(3) A contrasting finding was reported by Yandai *et al*, from Chad who reported lower resistance rates to most of the commonly tested antibiotics except for trimethoprim – sulfamethoxazole and Amoxicillin that reported 95.56% and 100%, respectively.(17) The high level resistance shown by this antibiotics especially Penicillin G, Amoxiclav and Ceftriaxone indicates that this antibiotics may no longer be effective as empiric therapy. Noticeable is the resistance displayed by ciprofloxacin and levofloxacin which are commonly used to treat UTI especially in outpatient. Amikacin and Meropenem were the most sensitive antibiotics (60% and 54.3%), respectively. This correlates well with the fact that the two antibiotics are not commonly used as compared to the aforementioned antibiotics. These two drugs are among the reserve drugs in the hospital and should be prescribed with caution to avoid further compounding the problem. Variation in the resistance patterns across different regions might be as a result of effective antimicrobial stewardship practices and infection prevention and control measures deployed at those regions.

The effectiveness of first-generation antimicrobial drugs has been greatly diminished in recent decades due to antibiotic resistance in *Klebsiella pneumoniae*. One of the main causes of antibiotic resistance is irrational antibiotic usage, which can also have detrimental effects on the efficacy of regional antibiotic resistance control initiatives in veterinary and human medicine. Some studies were able to posit that the presence of a capsule, which confers the mucoid nature on culture has a significant contribution to the resistance exhibited by *Klebsiella spp*. compared to non-mucoid isolates. The mucoid strains have a higher multiple antimicrobial index compared to the non-mucoid strains.(18)

Klebsiella pneumoniae is an established and significant pathogen in Healthcare Associated Infections MDR and extremely drug-resistant (XDR) *Klebsiella pneumoniae* are closely linked to ARGs ac- quired via plasmids and other MGEs, resulting in a “super resistome”. (19) The Multiple Antibiotic Resistant (MAR) index in this study ranges from 0.29 to 0.74; which is very high. An MAR index of 0.2 or higher indicates high level resistance suggesting that the isolate originated from an environment with high rate of antibiotic abuse. It also indicates possibility of plasmid-mediated resistance where antibiotics are frequently used indiscriminately which in turn confers high propensity and tendency for antibiotic resistance among the multidrug resistant bacterial isolates as supported by Bayode *et al*.(20) In addition to aiding in the appropriate treatment of patients, the results of the AST and MAR index will deter the careless use of antibiotics and stop the spread of bacterial drug resistance. The discovery of quinolone resistance genes in certain strains of *Klebsiella pneumoniae* had supported the idea that horizontal gene transfer is feasible and clarified why certain bacteria are multidrug resistant. Studies had confirmed that Multiple resistance genes may co-exist on the same isolates and the common genes implicated are the plasmid mediated.(21)(22) This is further complicated by the ability of multiple plasmids, depending on their incompatibility (Inc), to be harboured in a single CRE cell.(23) The global spread of various genes mediating antibiotic resistance was attributed to the presence of *IncF* plasmids, accounting for almost 40% (n = 254) of plasmicarbapenemases. *blaCTX-M*, *blaTEM*, *blaSHV*, *blaOXA-1/9*, *qnr*, and *aac-(6")-lb* were always identified co-existing with carbapenemases.(24)

In conclusion, *Klebsiella pneumoniae* represent a significant source of infections in our society. From mild to severe infections, it has been linked to both community and healthcare-associated infections. The hospital and the nation as a whole should be concerned about this organism's ability to resist antibiotics across multiple classes and generations. We suggested the creation of functioning antimicrobial surveillance committees that would be in charge of monitoring and accurately documenting the profile of resistance isolates and sending the information to the antimicrobial stewardship committee for remediable actions.

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