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Antibiotic Susceptibility of *Klebsiella pneumoniae* isolated from Selected Tertiary Hospitals in Osun State, Nigeria

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Abstract

Klebsiella pneumoniae is a pathogen of the *Enterobacteriaceae* family that causes healthcare-associated infections and has recently emerged as one of the most antibiotic-resistant organisms responsible for outbreaks in both community and healthcare settings. The aim of this study is to determine the resistance pattern of *Klebsiella pneumoniae* isolated from selected tertiary hospitals in Osun state, Nigeria. A total of 62 *Klebsiella pneumoniae* isolates were obtained from 1056 samples of urine, wound swab, ear swab, eye swab and other collection sites that were routinely submitted to the diagnostic laboratories of the selected tertiary hospitals. Susceptibility to twelve (12) antibiotics (Oxoid) was determined using the Kirby Bauer disk diffusion method for the 62 isolates. Rate of resistance to carbapenems, fluoroquinolones, polymyxins, monobactams, cephalosporins, penicillin and phosphonic acid derivative are 29.03%, 47.84%, 29.03%, 46.77%, 50.80%, 93.55%, and 37.10% respectively. The isolates were mostly susceptible to carbapenems, especially, Imipenem with 74.19%. Highest resistance was to Penicillin (93.55%). The multiple antibiotic resistance (MAR) index revealed that 52 (83.87%) out of 62 isolates were multi-drug resistant. Increase in antibiotic resistance continues to be a problem amidst patients infected with *Klebsiella pneumoniae* which can be most likely attributed to increase in antibiotic misapplication, misuse and abuse which is most prevalent among youths. It is therefore of utmost importance that consistent monitoring of antibiotic resistance be done as it will assist in the appropriate selection of empiric antibiotic treatment in the proper setting.

Keywords: *Klebsiella pneumoniae;* Resistance pattern; Tertiary hospitals.

1. Introduction

Klebsiella pneumoniae is a pathogen of the *Enterobacteriaceae* family that causes healthcare-associated infections and has recently emerged as one of the most antibiotic-resistant organisms responsible for outbreaks in both community and healthcare settings [1]. It has the ability to colonize gastrointestinal tract, nasopharynx, and the skin [2] and also capable of causing diverse infections ranging from minor infections to very critical ones such as urinary tract infections, soft tissue infection, intra-abdominal infection, septicaemia, wound or blood infections and pneumonia [3, 4].
Combating infections posed by this organism can mostly be done as a result of effective antimicrobial therapy. This involves administering of antibiotics to the infected patients. The high rate at which antibiotics are used all around the world in human therapy, animal therapy, and in livestock has given

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rise to the emergence of antibiotic-resistant isolates, leading to very detrimental problems in the affected individuals and in the community at large [5], as the treatment outcome of the infection would be affected by the presence of drug resistance in the infecting pathogen [4]. This however, can lead to prolonged stay in the hospital [6], cause a drastic increase in health care costs and in many cases, results in life-threatening and untreatable infections [7].

This study therefore aimed at determining the drug susceptibility of *Klebsiella pneumoniae* isolated from selected tertiary hospitals in Osun state, Nigeria, so as to assist in the appropriate selection of empiric antibiotic treatment for infections caused by *Klebsiella pneumoniae.*

2. Materials and methods

2.1 Study Location

This study was carried out in some selected hospitals in Osun State, Nigeria. Osun state lies approximately on Latitude 40°N of the equator and Longitude 7.34°E of the Greenwich meridian and about 1,100m above the sea level.

2.2 Collection of clinical isolates between September 2018 and February, 2019

A total of 62 *Klebsiella pneumoniae* isolates were obtained from 1056 samples of urine, wound swab, ear swab, eye swab and other collection sites that are routinely submitted by both male and female patients to the diagnostic laboratories of Wesley Guild Hospital, Ilesha, State Specialist Hospital, Asubiaro, Osogbo, LAUTECH Teaching Hospital, Osogbo and OAUTHC, Ile-Ife. The isolates were transported at 4°C using ice packs to the Microbiology laboratory, Adeleke University, Ede, Osun state where they were processed immediately. An ethical approval/clearance certificate with protocol number **LTH/EC/2019/01/397** was obtained from Ladoke Akintola University of Technology (LAUTECH) Research Ethics Committee to execute this research.

2.3 Conventional identification of Klebsiella pneumoniae isolates

Klebsiella pneumoniae isolates were identified using the conventional method described by Cheesbrough, [8]. This involved carrying out gram staining test, catalase test, urease test, oxidase test and coagulase test on the pre-identified *Klebsiella pneumoniae* colonies.

2.4 Antimicrobial susceptibility testing

Isolates confirmed as *Klebsiella pneumoniae* were tested for antimicrobial susceptibility by the Kirby-Bauer disk diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India, MV1084), according to the Clinical and Laboratory Standards Institute guidelines. Inoculated plates were incubated aerobically at 37 °C for 18-24h. Results were interpreted in accordance with criteria provided by CLSI [9]. *Pseudomonas aeruginosa* -Strain RH 815 (ATCC 10145) was used as a control which was tested on a weekly basis. The tested antibiotics were Imipenem (10 µg), Ertapenem (10 µg), Meropenem (10 µg), Cefepime (30 µg), Ceftazidime (30 µg), Penicillin (10 µg), Levofloxacin (10 µg), Norfloxacin (10 µg), Fosfomycin (200 µg), Moxifloxacin (5 µg), Aztreonam (10 µg) and Colistin sulphate (10 µg). Susceptibility of *Klebsiella pneumoniae* isolates to each antimicrobial agent was measured and categorized as sensitive, intermediate or resistant.

2.5 Analysis and Calculation of multiple antibiotic resistance (MAR) index

MAR index was calculated by following the method previously used by Osundiya *et al.* [10]. Data were entered into Microsoft Excel (Microsoft Corp., Redmond, WA) and analyses were done using Epi-info v.7.0

3. Results and Discussion

3.1 Distribution of Klebsiella pneumoniae isolated from selected hospitals in Osun state.

A total of 1056 samples of urine, wound swab, ear swab, eye swab and other sample types from the diagnostic laboratories of the selected hospitals were received between September, 2018 and February, 2019. Overall, *K. pneumoniae* was cultured from 62 (5.87%) of the1056 samples including 26(41.94%) from urine, 12(19.35%) from wound swab, 8(12.90%) from sputum, 3(4.83%) from ear swab, 2(3.22%) from HVS/ECS, 1(1.61%) from eye swab and throat, 3(4.83%) from blood, and 6(9.67%) from others. *Klebsiella pneumoniae* infected patients consisted of 33 males (53.23 %) and 29 females (46.77%). The percentage ratio of in -patient and out-patient examined were 54.84% and 45.16% respectively. The percentage distribution of the admission class for medical and surgical was 58.33% and 41.67% respectively. The highest incidence of *Klebsiella pneumoniae* was from patients with urine infections (41.94%). Maximum number of cases were from the age group less than 50 years (n= 43, 69.35%).

3.2 Antimicrobial susceptibility testing

Disk diffusion susceptibility results were available for the 62 isolates. Table-1 shows the antibiotic susceptibility profile of studied *Klebsiella pneumoniae* isolates. According to the invitro antibiotic susceptibility testing (AST), Imipenem was the most effective antibiotic against *Klebsiella pneumoniae* isolates (74.19% isolates were susceptible) and penicillin was the least effective antibiotics (93.55% isolates were resistant), (Table-1).

Susceptibility rates for Imipenem, Ertapanem and Meropenem (the carbapenem group of antibiotics tested) were 74.19%, 43.55% and 27.42% respectively and the susceptibility rates of other antibiotics tested including Levofloxacin, Colistin sulphate, Aztreonam, Ceftazidime, Cefepime, Penicillin, Moxifloxacin, Fosfomycin and Norfloxacin are 14.52%, 0.00%, 24.19%, 12.90%, 20.97%, 1.61%, 24.19%, 14.52% and 26.23% respectively. None of the *Klebsiella pneumoniae* isolates were susceptible to Colistin sulphate instead, a higher percentage of the isolates (70.97%) showed intermediate susceptibility to colistin while a higher percentage of the isolates showed resistance to Penicillin (93.55%).

Major pathogens such as *Klebsiella pneumoniae* have been implicated in health care associated infections (HAI). Treatment of these infections with first line antibioticsis becoming more difficult as a result of increasing rates of antibiotic resistance [10, 11]. Emerging and increasing rate of resistance to much newer antibiotics and otherwise efficacious antibiotics have resulted into full blown problems, especially as there is insufficient development of new drugs [10-12]. From this study, isolates of *Klebsiella pneumoniae* demonstrated highest level of susceptibility to Imipenem (74.19%), followed by Ertapenem (43.55%) and Meropenem (27.42%) which all belong to the carbapenem antibiotic class, however, some of the isolates still demonstrated resistance to antibiotics in this class. High level of susceptibility to Imipenem observed in this study corroborates the results obtained in a study carried out by Osundiya *et al.* [10], where 94.15% susceptibility to Imipenem was observed.

Penicillin was the least effective antibiotics as 93.55% of *Klebsiella pneumoniae* isolates showed resistance to it. The obviously visible high rate of resistance of isolates to penicillin is in tandem with the documented rate at which penicillinase producing *B-lactamases* strains are increasing rapidly among these organisms [13]. Figure-1 shows the antibiotic sensitivity pattern of *Klebsiella pneumoniae* isolates.

Overall, the resistance rate among strains of *Klebsiella pneumoniae* isolated from urine was higher than the other isolates as at least one of the isolates demonstrated resistance to the entire 12 antibiotics tested, while the least resistance rate was observed among the strain isolated from HVS/ECS as resistance was shown to just 4 out the 12 antibiotics tested.

Table 1-Antibiotic susceptibility profile of the *Klebsiella pneumoniae* isolates

Antibiotics class	**Antibiotics**	**Percentage (%) response of isolates to antibiotics**		
		No of Susceptible isolates	**No of Intermediate isolates**	**No of Resistant isolates**
Carbapenems	Meropenem(10µg)	17(27.42)	19(30.65)	26(41.94)
	Ertapanem (10µg)	27(43.55)	8(12.90)	27(43.55)
	Imipenem (10µg)	46(74.19)	15(24.19)	1(1.61)
Fluoroquinolones	Levofloxacin (10µg)	9(14.52)	20(32.26)	33(53.23)
	Norfloxacin (10µg)	16(26.23)	17(27.87)	28(45.90)
	Moxifloxacin (5µg)	15(24.19)	19(30.65)	28(45.16)
Polymyxins	Colistin sulphate (10µg)	0(0.00)	44(70.97)	18(29.03)
Monobactams	Aztreonam (10µg)	15(24.19)	18(29.03)	29(46.77)
Cephalosporins	Ceftazidime (30µg)	16(25.81)	12(19.35)	34(54.84)
	Cefepime (30µg)	13(20.97)	20(32.26)	29(46.77)
Penicillin	Penicillin (10µg)	1(1.61)	3(4.84)	58(93.55)
Phosphonic acid derivative	Fosfomycin (200µg)	9(14.52)	30(48.39)	23(37.10)

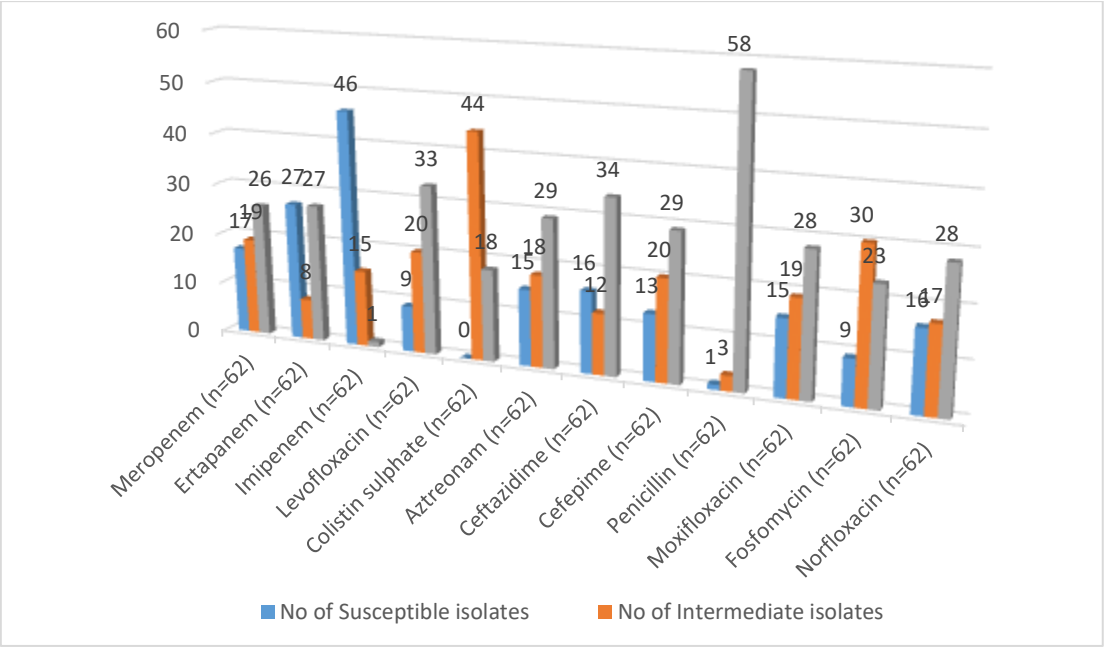

Figure 1-Antimicrobial susceptibility pattern of *Klebsiella pneumoniae* isolates

3.3 Multiple antibiotic resistances (MAR) index

Results of multiple antibiotic resistances (MAR) index of *Klebsiella pneumoniae* isolates are as shown in Table-2.

Table 2-Multiple antibiotic resistances (MAR) index of *Klebsiella pneumoniae* isolates

ISOLATE CODE	ANTIBIOTIC RESISTANCE PATTERN	MAR INDEX
[a]K1	LEV, MXF, NOR, P	0.33
[a]K2	LEV, MXF, NOR, P	0.33
[a]K3	CT, FOT, LEV, MXF, P	0.41
[a]K4	ATM, CAZ, CT, FOT, LEV	0.41
[a]K5	ATM, CAZ, LEV, P	0.33
[a]K6	P	0.08
[a]K7	ATM, CAZ, FOT, P	0.33
[a]K8	P	0.08
[a]K9	CAZ, FEP, ETP, IPM, LEV, MEM, NOR, P	0.66
[a]K10	LEV, MXF, NOR	0.25
[a]K11	CT, P	0.17
[a]K13	FEP, FOT, LEV, MXF, NOR, P	0.50
[a]K14	FOT, LEV, MXF, NOR, P	0.42
[a]K15	LEV, MXF, NOR, P	0.33
[a]K16	CAZ, CT, FOT, P	0.33
[a]K17	FEP, MXF, P	0.25
[a]K18	CT, FOT, LEV, MXF. P	0.42
[a]K19	ATM, CAZ, FEP, CT, P	0.42

[a]K20	FEP, CT, P	0.25
[a]K21	ATM, P, TIC	0.25
[a]K22	P	0.08
[a]K23	LEV, MXF, NOR, P	0.33
[a]K24	ATM, FEP, CT, ETP, FOT, MEM, P	0.58
[a]K25	ATM, CAZ. FEP, CT, ETP, FOT, MEM, P	0.66
[a]K26	CAZ, FEP, CT, ETP, FOT, LEV, MEM, P	0.66
[a]K27	CAZ, FEP, CT, ETP, FOT, MEM, P	0.58
[a]K28	ATM, CAZ, FEP, ETP, FOT, LEV, MEM, MXF, NOR, P	0.83
[a]K29	ATM, CAZ, FEP, CT, ETP, FOT, MEM, P	0.66
[a]K30	CAZ, ETP, FOT, MEM, P	0.42
[a]K31	ATM, CAZ, FEP, ETP, MEM, MXF, NOR, P	0.66
[a]K32	ATM, CAZ, FEP, ETP, FOT, MEM	0.50
[a]K33	CT, ETP, FOT, MEM, P	0.42
[b]K1	CAZ, FOT, P	0.25
[b]K2	CAZ, FEP, CT, ETP, FOT, LEV, MEM, NOR, P	0.75
[b]K3	CAZ, CT, FOT, P	0.33
[b]K4	CAZ, CT, FOT, LEV, P	0.42
[b]K5	P	0.08
[b]K6	ATM, CAZ, LEV, MXF, NOR, P	0.50
[b]K7	LEV, MXF, NOR, P	0.33
[c]K1	P	0.08
[c]K2	ATM, LEV, MXF, NOR, P	0.42
[c]K3	P	0.08
[c]K4	P	0.08
[c]K5	P	0.08
[c]K6	P	0.08
[c]K7	ATM, FEP, LEV, NOR, P	0.42
[c]K8	ATM, CAZ, CT, FOT	0.33
[d]K1	ATM, CAZ, FEP, ETP, LEV, MEM, MXF, NOR, P	0.75
[d]K2	ATM, CAZ, FEP, ETP, LEV, MEM, MXF, NOR, P	0.75
[d]K3	ATM, CAZ, FEP, ETP, LEV, MXF, NOR, P	0.66
[d]K4	ATM, CAZ, FEP, ETP, LEV, MEM, MXF, NOR, P	0.75
[d]K5	ATM, CAZ, FEP, ETP, LEV, MEM, MXF, NOR, P	0.75
[d]K6	ATM, CAZ, FEP, ETP, LEV, MEM, MXF, NOR, P	0.75
[d]K7	ATM, CAZ, FEP, ETP, FOT, LEV, MEM, MXF, NOR, P	0.83
[d]K8	ATM, CAZ, FEP, ETP, LEV, MEM, MXF, NOR, P	0.75
[d]K9	ATM, CAZ, FEP, ETP, MEM, P	0.50

[d]K10	ATM, CAZ, FEP, ETP, FOT, LEV, MEM, MXF, NOR, P	0.83
[d]K11	ATM, CAZ, FEP, ETP, LEV, MEM, MXF, NOR, P	0.75
[d]K12	ATM, CAZ, FEP, ETP, LEV, MEM, MXF, NOR, P	0.75
[d]K13	ATM, CAZ, FEP, CT, ETP, LEV, MEM, MXF, NOR, P	0.83
[d]K14	ETP, MEM, P	0.25
[d]K15	ATM, CAZ, FEP, ETP, LEV, MEM, MXF, NOR, P	0.75

Key: MEM= Meropenem, ETP= Ertapanem, IPM= Imipenem, LEV= Levofloxacin, CT= Colistin sulphate, ATM= Aztreonam, CAZ= Ceftazidime, FEP= Cefepime, P= Penicillin, MXF= Moxifloxacin, FOS= Fosfomycin, NOR= Norfloxacin

[a] = isolates collected from Wesley Guild Hospital, Ilesha, [b] = isolates collected from LAUTECH, Osogbo, [c] = isolates collected from Asubiaro, Osogbo, [d] = isolates collected from OAUTHC, Ife

In total, 52 (83.87%) of 62 *Klebsiella pneumoniae* isolates were identified as multidrug resistant (MDR), having MAR index greater than 0.2.

The rate at which multi-drug resistance is increasing in many parts of the world is alarming which ultimately poses a serious therapeutic dilemma [14]. Analysis of the multiple antibiotic resistance (MAR) index of *Klebsiella pneumoniae* isolates in this study showed that 59 (95.16%) out of 62 isolates demonstrated resistance to not less than three antibiotics out of the twelve antibiotics that were tested. This corroborates the results obtained from the study carried out by Osundiya *et al.* [10], where very high rate of multidrug resistance was observed in the *Klebsiella pneumoniae* isolates tested. This, however was higher than what was observed in a study carried out by Olayinka *et al.* [15]. This could be due to the difference in defining what multidrug resistance connotes as their estimation was done on the basis of isolate's resistance to not less than four antibiotics whereas, three were used in this study as it is much closer with the general definition of multi-drug resistance according to Lambert, [16].

Also, results from a study carried out by Subha and Ananthan, [17] reported the rate of multi-drug resistance of isolates of *K. pneumoniae* as 100%. This corroborates the result of this study where 95.16% of the isolates were multidrug resistant. The increasing prevalence of multi drug resistant strains of *K. pneumoniae* which has not stopped existing overtime shows that the antibiotics used in treatment of various infections are yet to be administered correctly and there are possibilities of irrational use of antibiotics or resistant genes transfer between organisms by means of plasmids capable of taking up resistant genes, integrons, bacteriophages and transposons [18- 20]. In-vitro sensitivity of antibiotics is an important factor that should be seriously considered in selecting the antimicrobial agents for treatment of an infection [7].

Conclusion

Increase in antibiotic resistance continues to be a problem amidst patients infected with *Klebsiella pneumoniae* which can be most likely attributed to increase in antibiotic use. It is therefore of utmost importance that consistent monitoring of antibiotic resistance be done as it will assist in the appropriate selection of empiric antibiotic treatment in the proper setting.

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