

RESEARCH ARTICLE

Prevalence of Multi-Antibiotic Resistant *Escherichia coli* and *Klebsiella* species obtained from a Tertiary Medical Institution in Oyo State, Nigeria

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ABSTRACT

Background: The development of multi-antibiotic resistant bacteria, especially Gram-negative bacteria which are the major cause of hospital-acquired infections worldwide, had been increasing. *Escherichia coli* and *Klebsiella* sp. had become more resistant to different classes of antibiotics, and the treatment of infections caused by these bacteria had developed into a challenge in both developed and developing countries. This study had determined the multi-antibiotic resistance (MAR) patterns of *Escherichia coli* and *Klebsiella* sp. isolated from clinical inpatient and outpatient samples.

Method: The present study had used 50 *E. coli* and 48 *Klebsiella* sp. isolates. Antibiotic susceptibility test had been carried out by using disk diffusion method, and the interpretation of results of the zones of inhibition had accorded with Clinical Laboratory Standards Institute (CLSI). The antibiotics used had included the following: streptomycin, ciprofloxacin, erythromycin, nitrofurantoin, amikacin, gentamicin, ofloxacin, cefepime, oxacillin, colistin sulfate, cefotaxime, ceftazidime, pefloxacin, and cloxacillin. **Results:** *E. coli* and *K. pneumoniae* had shown high-resistance patterns. *E. coli* had exhibited high resistance against cloxacillin (96%), oxacillin (96%), erythromycin (88%), and most especially streptomycin (98%). Similarly, *K. pneumoniae* had presented a high resistance to streptomycin (88%), cloxacillin (92%), oxacillin (92%), and colistin (92%). *E. coli* had presented the highest multidrug resistance with a MAR index of 1.00. A total of 17 *E. coli* isolates had shown resistance to the 14 antibiotics tested. **Conclusion:** *E. coli* and *Klebsiella* sp. in clinical isolates in outpatients and inpatients in Ibadan, Western Nigeria had demonstrated high antimicrobial resistance.

Thus, such condition should be considered a major public health concern, and measures must be taken to establish the sources and drivers of this problem.

Keywords: Antibiotics Resistance, *Escherichia coli*, *Klebsiella* sp., Nigeria

INTRODUCTION

Antibiotic resistance (AR) is a global problem especially in the developing countries; this condition increasingly compromises the outcome of various infections that were until recently, treatable and remain the common infections in Africa.¹ AR also threatens public health in both developed and developing countries.² The development of resistance by bacteria to different antibiotics has resulted in the difficult treatment of infectious diseases. The infections caused by antibiotic resistant bacteria, most especially multi-resistance bacteria, can lead to serious health problems, such as long hospital stay, treatment failure, and death.³ The major factors causing the development of bacterial resistance include the indiscriminate use of antimicrobial agents in human and animal medicine, agriculture, and aquatic farming. Different mechanisms or their combination are used by bacteria in developing resistance to antibiotics; however, a particular mechanism may be dominant and can be identifiable.⁴ The presence of plasmids that contain one or more resistance genes, with each encoding a single AR phenotype, often causes the development of multiple AR (MAR) in bacteria.⁵ These AR genes can transfer to other bacteria of the same or different species.

MAR index is an effective, valid, and cost-effective method that is used in source tracking of antibiotic resistant organisms.⁶ 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. A MAR greater than 0.2 means that the high risk source of contamination is where antibiotics are frequently used.⁷

Escherichia coli is a Gram-negative, motile facultative anaerobic bacterium which may or may not be encapsulated. This species causes a wide range of infections in humans and is the most common causative agent in urinary tract infections. This bacterium is a commonly isolated in medical microbiology laboratories.¹ *E. coli* is abundant in the human gastro-intestinal tract and also serves as a reservoir or carrier of antibiotic resistant genes.⁸ *E. coli* shows increasing resistance to different antibiotics especially to third generation cephalosporins.⁹

Klebsiella species are Gram-negative, rod shaped, nonmotile, facultative aerobic and anaerobic bacteria. *Klebsiella* sp. are an emerging threat to human health that causes both endemic and epidemic infections. Most hospital-acquired infections caused by *Klebsiella* sp. cause a challenge in their treatment because of the development of MAR strains. The resistance of *Klebsiella* strains to different generations of cephalosporins, especially the third generation, was first reported in 1981; since then, these bacteria have become more resistant to antibiotics.¹⁰

This study was carried out to determine the prevalence of multi-antibiotic resistant *E. coli* and *Klebsiella* sp. from clinical isolates.

METHODOLOGY

Collection of bacterial isolates

Preidentified 98 clinical isolates of Enterobacteriaceae were isolated from blood samples of both outpatients

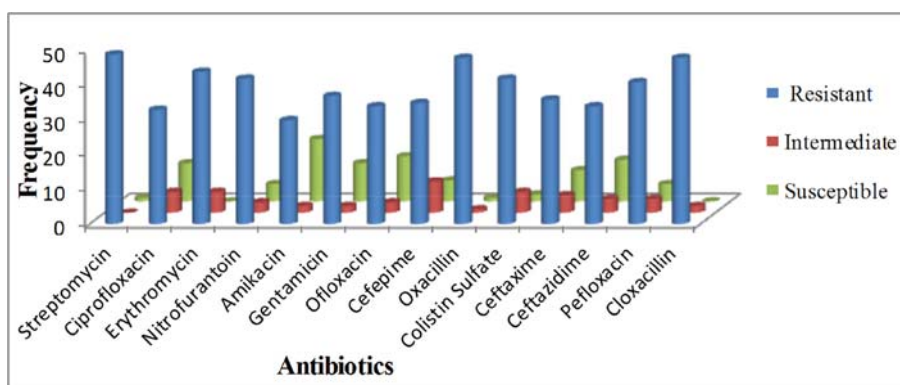


Figure 1. Comparison of sensitivity patterns to different antibiotics of *E. coli* isolated from inpatients and outpatients.

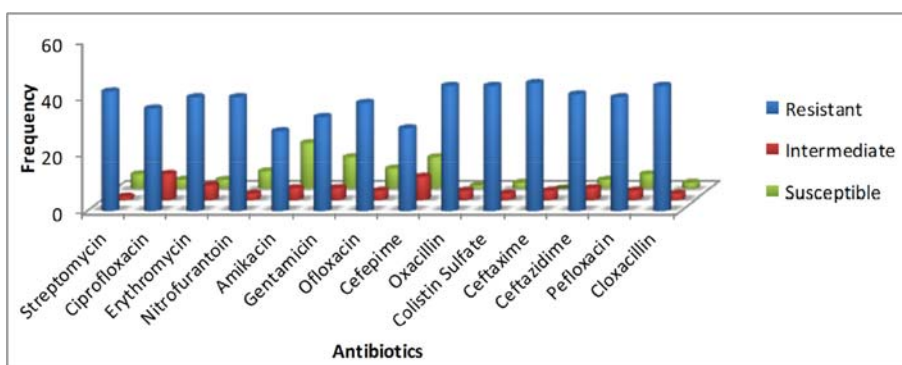


Figure 2. Comparison of sensitivity patterns to different antibiotics of *Klebsiella* sp. isolated from inpatients and outpatients.

and inpatients from University College Hospital, Ibadan and had been in storage for less than a month. The present study examined 50 *Escherichia coli* and 48 *Klebsiella* sp. samples. Nutrient agar slant bottles contained the isolates were stored. The bacterial isolates were then transferred to Microbiology Laboratory of Pure and Applied Biology, Ladoko Akintola University of Technology, Oyo state, Nigeria for further study.

Antibiotic susceptibility test

Antibiotic susceptibility test was carried out by using 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 nutrient agar plates. The plates contained the impregnated

antibiotics discs (Oxoid, UK), which were incubated at 37 °C for 24 h. The experiment used 14 antibiotics: streptomycin (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), pefloxacin (5 µg), ofloxacin (5 µg), erythromycin (15 µg), nitrofurantoin (300 µg), oxacillin (50 µg), cloxacillin (10 µg), colistin sulfate (25 µg), cefepime (5 µg), ceftazidime (30 µg), and cefotaxime (75 µg). Zones of diameter were measured and interpreted as susceptible, intermediate, and resistant in accordance with the recommendation of Clinical and Laboratory Standard Institute.¹¹

Determination of MAR index

Determination of MAR index followed the procedure described by (5), in which the number of antibiotics an isolate is resistant to (a) is divided by the total number

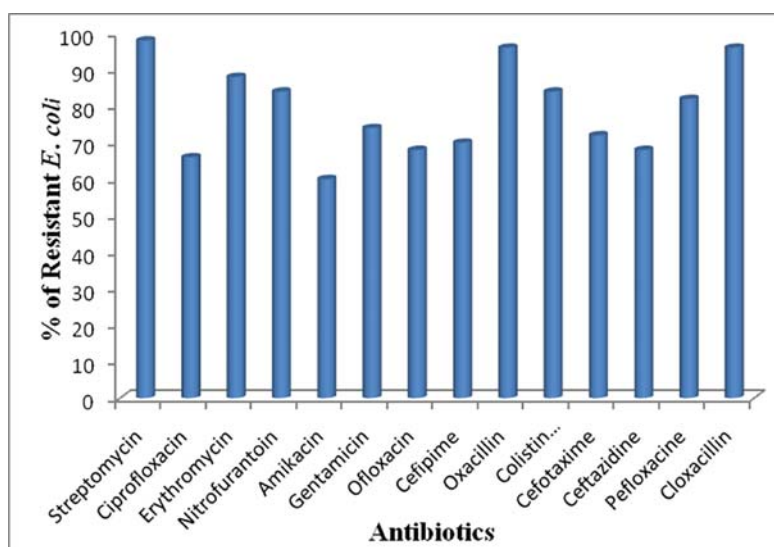


Figure 3. Percentage of *E. coli* resistant to different antibiotics used in this study.

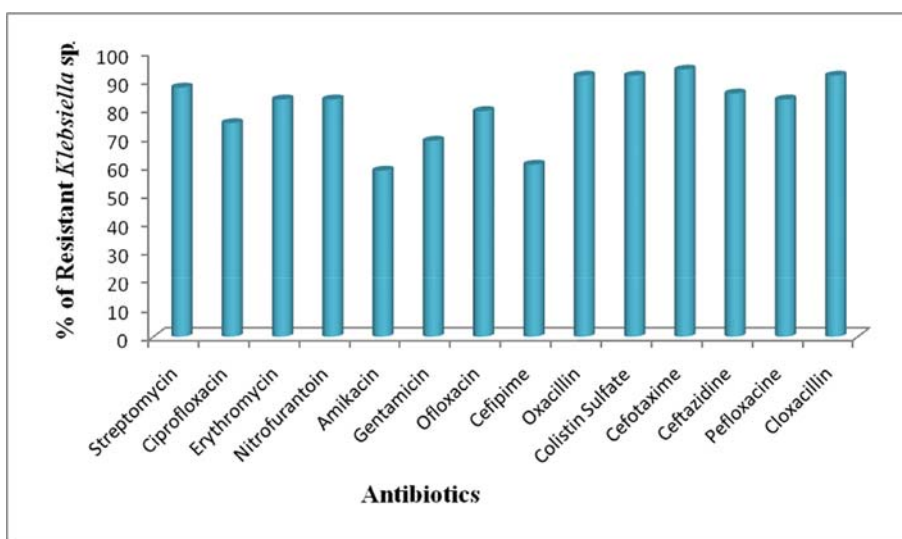


Figure 4. Percentage of *Klebsiella* sp. resistant to different antibiotics used in this study.

of the antibiotics used in the study (b). The calculating formula is shown below:

$$\text{MARIndex} = a/b$$

Statistical analysis

Data analysis used descriptive statistics, frequencies, and bivariate analyses (cross tabulations).

RESULTS

The antibiotic susceptibility profiles of *E. coli* and *Klebsiella* strains were classified as "Resistant," "Intermediate," and "Susceptible" (Figures 1 and 2, respectively). The test of all the strains against 14 different antibiotics applied the Kirby – Bauer disc diffusion and underwent standardization and evaluation by the methods of the National Committee for

the Clinical Laboratory Standards. The percentile distribution was obtained for the prevalence of resistance of isolates to different classes of antibiotics, including penicillin (oxacillin, cloxacillin), aminoglycosides (gentamicin, streptomycin, and amikacin), fluoroquinolones (ciprofloxacin, ofloxacin, and pefloxacin), macrolides (erythromycin), nitrofurantoin, extended spectrum cephalosporins (cefepime, ceftazidime, and cefotaxime), and colistin sulfate (Figures 3 and 4). Comparison focused on the resistance patterns between *E. coli* and *Klebsiella* (Figure 5). Table 1 shows the MAR indices of both *E. coli* isolates and *Klebsiella*.

DISCUSSION

The findings in this study reveal the high resistance rates of strains of *Escherichia coli* and *Klebsiella* sp. to

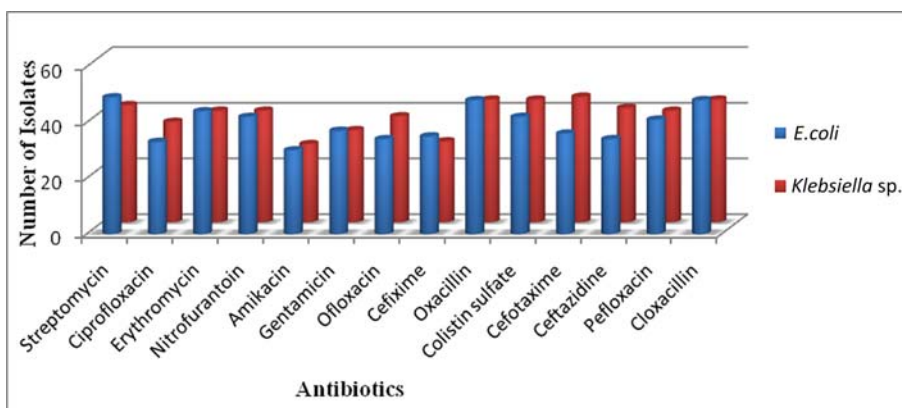


Figure 5. Comparison of resistance of *E. coli* and *Klebsiella* sp. to different antibiotics.

Table 1. Frequency of resistance bacterial isolates and the MAR index.

<i>Escherichia coli</i>	<i>Klebsiella</i> spp.
No of Antibiotics No of Isolates MAR Index	No of Antibiotics No of Isolates MAR Index
Resistant to	Resistant to
1 0 -	1 0 -
2 0 -	2 0 -
3 1 0.21	3 0 -
4 1 0.29	4 1 0.29
5 0 -	5 0 -
6 0 -	6 1 0.43
7 2 0.50	7 1 0.50
8 5 0.57	8 2 0.57
9 5 0.64	9 3 0.64
10 6 0.71	10 10 0.71
11 6 0.79	11 4 0.79
12 4 0.86	12 7 0.86
13 3 0.93	13 12 0.93
14 17 1.00	14 7 1.00
Total 50	48

commonly used antibiotics, such as streptomycin (98%), cloxacillin and oxacillin (96%), and colistin (84%), with relatively lower resistance rates to ciprofloxacin (66%); these findings are similar to those from neighboring countries, namely Kenya¹² and Uganda.¹³ The findings can possibly result from the association of the isolates with AmpC enzymes. *E. coli* showed the highest resistance toward streptomycin, followed closely by cloxacillin, oxacillin, and erythromycin. However, the clinical isolates exhibited susceptibility to amikacin (36%), followed closely by ofloxacin (26%), cefotaxime (18%), gentamycin, and ciprofloxacin (22%). The susceptibility patterns are similar to those observed by Nahla et al.¹⁴ *Klebsiella* presented the highest resistance toward cefotaxime (93.75%), followed closely by cloxacillin, oxacillin, and colistin sulfate

(91.67%). A considerable resistance occurred with streptomycin, similar to that in *E. coli* and as shown in the work of Kim et al.¹⁵ The present work revealed high susceptibility rates toward amikacin (33.37%), which is similar to the result found in *E. coli*, followed by gentamycin and cefepime (22.92%). This study also revealed that the highest multidrug resistance occurred in *E. coli* with MAR index of 1.00, and 17 out of 50 isolates (34%) were resistant to all 14 antibiotics. The MAR index of *Klebsiella* sp. was 0.93, which differs from that of another work⁵ that reported an index of 0.4; contrary to the low resistance to aminoglycosides reported previously, this work reported resistance of 87.5%, 68.75%, and 58.38% in streptomycin, gentamycin, and amikacin, respectively.

For *Klebsiella* sp., 7 isolates (14.6%) were resistant to all 14 antibiotics, whereas 12 isolates (25%) were resistant to 13 out of 14 antibiotics tested. These findings are similar to the results of Osundiya et al.⁵ where 66.7% of the *Klebsiella* isolates achieved a MAR index greater than 0.2.

The susceptibility of *E. coli* to fluoroquinolones approximated 80%, and this finding correlates with a review¹⁶ that reported an average of 80% susceptibility across different continents. In Khalit et al.¹⁷ *Klebsiella* was resistant to ampicillins and third generation cephalosporins, which corroborates the findings of this research.

CONCLUSION

These findings have implications for the choice of antibiotics for empirical management of infections and effective hospital infection control. Effective control must be in place to limit the spread of these pathogens. Further work should examine and identify the genes responsible for resistance and/or virulence in these organisms to device preventive and curative alternative therapies in the form of vaccines and antimicrobials, respectively.

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