



Antimicrobial Susceptibility Pattern of *Klebsiella pneumonia* and *Escherichia coli* Isolated from Stool Samples of Patients in Two Tertiary Hospitals in Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author SID designed the study. Author NPA performed the statistical analysis. Author NCK managed the analyses of the study, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Reduced susceptibility of antibiotics against Enterobacterial strains have emerged as an important public health problem worldwide. Infections caused by *Escherichia coli* and *Klebsiella pneumonia* can affect severely ill patients, and their colonization of human gut, endangers population at large in communities, and in hospitals. This research is aimed at determining the susceptibility pattern of *Escherichia coli* and *Klebsiella pneumonia* from stool of patients in two tertiary hospitals in Rivers State, Nigeria. A total of 114 stool samples were collected from patients. Stool samples were collected in sterile biological specimen bottles and were sent to the laboratory immediately after collection. Stool samples were inoculated by streaking on Eosin methylene blue and MacConkey agar plates. Isolates were characterized using standard microbiological methods and were stored and used for further tests. The result showed that nineteen isolates of *E. coli* were 100% resistant to Cefuroxime and Augmentin, while 78.9%, 68.4% and 42.1% were resistant to cefixime,

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Ceftazidime and Nitrofurantoin, respectively. The result for the susceptibility pattern of the *Klebsiella* isolates showed 100% resistance to cefuroxime and Augmentin. Resistance to ceftazidime, cefixime and nitrofurantoin were observed to be 70%, 60% and 45%, respectively. Isolates of *E. coli* and *Klebsiella* were highly susceptible to Meropenem and ofloxacin. The isolates of *E. coli* and *Klebsiella* showed multi-drug resistance to the different antibiotics. Although the meropenem, ofloxacin and ciprofloxacin antibiotics showed high level of sensitivity to these isolates, there were still some level of resistance recorded.

Keywords: Antimicrobial susceptibility; *Klebsiella*; *E. coli*; stool samples; carbapenem-resistant Enterobacteriaceae.

1. INTRODUCTION

With an increase in the number of individuals being presented to anti-microbials, the intestinal microflora faces consistent weight of anti-microbial determination, which has brought about the development of strains that are resistant to different types of antibiotics including carbapenem-resistant strains. This could represent a serious issue as intestinal Enterobacteriaceae are most generally embroiled in human contaminations and antibiotic options in infections caused by carbapenem-resistant Enterobacteriaceae (*E. coli* and *Klebsiella spp.*) may be limited to colistin, tigecycline and Polymyxin B. Routine laboratory culturing of feces tests for diagnosing normal clinical pathogens may frequently disregard commensal Enterobacteriaceae that can harbor safe phenotypes. Anti-microbial abuse and inappropriate sanitation and cleanliness in urban ghetto (slums) zones can prompt the quick spread and huge scope carriage of multi-or container medicate safe secludes in the intestinal microbiota that can be a potential reason for endogenous and exogenous infections[1]. Bacteria have evolved diverse and remarkable ways to avoid antimicrobials in several cases, resistance is due to a minor structural alteration in the target so that it is no longer bound by the drug yet still functions [2]. For example, streptomycin normally binds to a part of prokaryotic 30S ribosomal subunit that is critical for protein synthesis. A slight alteration in the structure of ribosome result in a distortion, so that streptomycin is no longer able to bind but the ribosome can still functionally translate mRNA. Alteration in membrane permeability or its other function may confer antibiotic resistance [3]. To determine whether an organism is sensitive or not, a culture of the organism is spread over a surface of an agar medium, and an antibiotics disk containing a precise amount of antibiotic is placed on top of it, following incubation, the zone of inhibited growth is measured and if it is large enough, the organism is called sensitive.

A complete profile which displays the susceptibility result of microorganisms to respective antibiotics is referred to as an antibiogram [4]. The antibiogram is very much important since it is able to inform the public health microbiologist or clinicians the antibiotics which are able to fight or inhibit a particular disease-causing pathogen since microbial isolates have devised various methods of resisting commonly used drugs [5].

So as to invert the disturbing pattern of an expanding antimicrobial obstruction, doctors just as the overall population must assume greater liability for the suitable utilization of these lifesaving drugs. Physicians need to increase their effort to identify the causative agent of infectious diseases and only if appropriate, prescribe suitable antimicrobials, they must also educate their patients about the use of prescribed drugs in order to increase patient compliance [6]. Patients, meanwhile need to carefully follow the instructions that accompany their prescriptions even if those instructions seem inconvenient.

Antibiotics resistance occurs when organisms no longer respond to antibiotic actions designed to kill them. Enterobacteriaceae bacterial are finding a new way of avoiding the effect of antibiotics used in treatments of infections also known as being resistant to that particular antibiotics used [3]. For several years, antibiotic-resistant Enterobacteriaceae have increased significantly, being reported worldwide verification over the top expensive to treat particularly *Escherichia coli* and *Klebsiella pneumoniae* which are the most widely recognized pathogens related with sedate opposition and can show protection from various anti-infection agents even to the ongoing anti-infection agents [1]. The normal habitats for these pathogens include; the intestinal tract of humans and animals, which are frequently associated with serious nosocomial as well as community-acquired infections such as

pneumonia, sepsis, urinary tract infections, and intra-abdominal infections [2]. The emergence of carbapenem-resistant Enterobacteriaceae (*E. coli* and *Klebsiella*) is related with restricted remedial alternatives and expanded mortality in patients tainted by these strains [7]. These living beings likewise have the affinity to experience far reaching dispersal through portable hereditary components [8]. Enteric strains having these carbapenemases have indicated surprising accomplishment as large-scale geological scattering. Such strains consist primarily of *Klebsiella pneumoniae* and other members of Enterobacteriaceae such as *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Citrobacter spp.*, *Proteus spp.*, *Enterobacter spp.*, *Providencia spp.*, *Morganella spp.* which produce the serine carbapenemases, *K. pneumoniae* carbapenemase (KPC) or the metallo-beta-lactamases VIM or NDM-1 [9]. Gut colonization by CRE may act as a reservoir of these pathogens for dissemination within an enclosed setting as in a hospital [2] considering the emerging threat of these multidrug-resistant pathogens, the present study is to observe the prevalence of CRE in patients in hospitals using both phenotypic and genotypic methods. Meropenem is a novel carbapenem/ β -lactamase antimicrobial inhibitor. It exhibits effective antibacterial activity against CRE isolates [6] with susceptibility rates ranging from 66.2 to 100%.

This study was carried out to ascertain the antimicrobial susceptibility pattern of *Klebsiella pneumoniae* and *Escherichia coli* isolated from stool samples of patients in two tertiary hospitals in Port Harcourt, Rivers State, Nigeria.

2. METHODOLOGY

2.1 Study Area

This research study was carried out in two tertiary hospitals-the University of Port Harcourt Teaching Hospital and Save a Life Mission Hospital. The hospitals are located in Rivers State, Nigeria. The study was for a period of three months between January, 2019 to March 2019. Faecal samples were collected using sterile faecal sample bottles. The sterile containers were properly numbered and labelled, and also transferred into sterile bags before transporting them to microbiology laboratory in Rivers State University for analysis. A total of one hundred and fourteen (114) faecal samples were collected from (45) male and (69) female patients for a period of three months.

2.2 Isolation of *Klebsiella pneumoniae* and *Escherichia coli*

Klebsiella pneumoniae and *Escherichia coli* were isolated with Eosin methylene blue (EMB) and MacConkey (MA) agar using streak plate method according to Lancette and Bennett [10] and incubated for 18 to 24 hrs at 37°C and examined for growth. Characteristics colonies were described and subcultured onto nutrient agar plates and incubated for another 24 hours for pure cultures (for further tests) were then preserved in sterile 10% glycerol broth at 4°C. Gram stain reaction and biochemical tests Such as indole, catalase, methyl red, sugar fermentation and citrate tests were carried out according to Cheesbrough [11].

2.3 Antimicrobial Susceptibility Testing

Susceptibility testing was performed for all the identified clinical isolates according to the Clinical and Laboratory Standards Institute (CLSI) guideline using the Kirby-Bauer disc method. Using sterile wire loop, 3-5 colonies of the test organisms were emulsified into test-tubes containing 0.5 McFarland standard of normal saline. In a good light, the turbidity of suspension was matched with the turbidity of a turbidity standard (equivalent to 0.5 McFarland) prepared immediately before use. If there was no enough growth, the tube was incubated at 37°C for 2-4 hours or until it reached the turbidity of 0.5 McFarland standard.

Using sterile swab, a plate of Mueller Hinton agar (Oxoid, UK) prepared to manufacturer's instructions, was inoculated with the test organism. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension. The surface of the medium was streaked in three directions, rotating the plate approximately 360°C to ensure even distribution. With the petri-dish lid in place, 3-5 minutes was given to allow the surface of the agar to dry. Using sterile forceps, the respective antibiotic discs were placed onto the agar. Each disc was slightly pressed down to ensure its contact with the agar. Within 30 minutes of applying the discs, the plates were inverted and incubated at 37°C for 18 to 24hrs. After overnight incubation, the test plates were examined. Using a ruler on the underside of the plate, the diameter of each zone of inhibition was measured in mm, the end point of inhibition. The MAR index which represent the number of

antibiotics the isolates were resistant to were calculated as described by Akani et al. [5].

3. RESULTS AND DISCUSSION

Number of *E. coli* and *Klebsiella pneumoniae* isolates and their percentage occurrence by Sex is illustrated in Table 1. In Table 2, the resistance of *E. coli* to Cefuroxime and Augmentin were 100%. The isolates were also 78.9%, 68.4%, 42.1% and 10.5% resistant to Cefixime, Ceftazidime, Nitrofurantoin and Meropenem, respectively. Though no isolates of *E. coli* were completely resistant to Ofloxacin but intermediate response was 15.8%. The result also showed that *E. coli* were more susceptible to Meropenem with 89.5% followed by Ofloxacin 84.2%, Ciprofloxacin 73.7%, Gentamicin 68.4% and Ceftazidime 26.3%. On the contrary, Cefuroxime

and Augmentin was completely inactive to isolates of *Klebsiella spp.* Resistance of *Klebsiella* isolates to Ceftazidime was 70% while resistance to Meropenem, Gentamicin, and Ofloxacin 10% (Table 3). It was also observed that Meropenem were more effective against *Klebsiella spp.* with 90% susceptibility rate, followed by Ofloxacin 85%, Gentamicin 80%, and Nitrofurantoin 40% (Table 3).

The result for the Multiple antibiotic resistance (MAR) of *E. coli* and *Klebsiella spp* is illustrated in Table 4. The result showed that the isolates of *E. coli* and *Klebsiella spp* exhibited multiple antibiotic resistance. MAR index > 0.2 is reported to be an attribute of areas where such antibiotics are highly utilized which could have risen due to indiscriminate utilization of such antibiotics [5].

Table 1. Number of *E. coli* and *Klebsiella pneumoniae* isolates and their percentage occurrence by sex

Gender	No. of isolates	<i>E. coli</i>	<i>Klebsiella pneumoniae</i>
Male	20	7 (35.0%)	7 (35.0%)
Female	32	12 (37.5%)	13 (40.6%)
Total	52		19 (36.5%)

Table 2. Susceptibility pattern of *E. coli* to various antibiotics

Antibiotics	Concentration	Resistant n (%)	Intermediate	Susceptible
Ceftazidime	30µg	13 (68.4)	1 (5.3)	5 (26.3)
Cefuroxime	30µg	19 (100)	0 (0.0)	0 (0.0)
Gentamicin	10µg	3 (15.8)	3 (15.8)	13 (68.4)
Ofloxacin	5µg	0 (0.00)	3 (15.8)	16 (84.2)
Augmentin	30µg	19 (100)	0 (0.0)	0 (0.0)
Cefixime	5µg	15 (78.9)	1 (5.3)	3 (15.8)
Nitrofurantoin	30µg	8 (42.1)	1 (5.3)	10 (52.6)
Ciprofloxacin	5µg	2 (10.5)	3 (15.8)	14 (73.7)
Meropenem	10µg	2 (10.5)	0 (0.0)	17 (89.5)

Table 3. Susceptibility pattern of *Klebsiella spp.* to various antibiotics

Antibiotics	Concentration	Resistant n (%)	Intermediate	Susceptible
Ceftazidime	30µg	14 (70)	1 (5.0)	5 (25.0)
Cefuroxime	30µg	20 (100)	0 (0.0)	0 (0.0)
Gentamicin	10µg	2 (10.0)	2 (10.0)	16 (80.0)
Ofloxacin	5µg	2 (10)	1 (5.0)	17 (85.0)
Augmentin	30µg	20 (100)	0 (0.0)	0 (0.0)
Cefixime	5µg	12 (60)	2 (10)	6 (30.0)
Nitrofurantoin	30µg	9 (45)	3 (15)	8 (40)
Ciprofloxacin	5µg	4 (20)	4 (20)	12 (60)
Meropenem	10µg	2 (10)	0 (0.0)	18 (90)

Table 4. Mar index of *E. coli* and *Klebsiella pneumonia* isolates

MAR index	<i>E. coli</i> (19)	<i>Klebsiella</i> spp. (20)
0.0		
0.1	1 (5.2)	1 (5.0)
0.2	3 (15.8)	2 (10.0)
0.3	3 (15.8)	3 (15.0)
0.4	4 (21.0)	0.0 (0.0)
0.5	0.0 (0.0)	0.0 (0.0)
0.6	0.0 (0.0)	5 (25.0)
0.7	0.0 (0.0)	0.0 (0.0)
0.8	0.0 (0.0)	0.0 (0.0)
0.9	8 (42.2)	0.0 (0.0)
1.0	0.0 (0.0)	9 (45.0)

From the result obtained, it was observed that *E. coli* were resistant to Cefuroxime and Augmentin but susceptible to Meropenem, Ofloxacin, Ciprofloxacin and Gentamycin. *Klebsiella* spare susceptible to Gentamicin, meropenem and Ofloxacin while Cefuroxime and Augmentin were not effective. In order to reverse the alarming trend of an increasing antimicrobial resistance, physicians as well as the general public must take more responsibility for the appropriate use of these lifesaving drugs. Physicians need to increase their effort to identify the causative agent of infectious diseases and only if appropriate, prescribed suitable antimicrobials, they must also educate their patients about the use of prescribed drugs in order to increase patient compliance. Patients, meanwhile need to carefully follow the instructions that accompany their prescriptions even if those instructions seem inconvenient. Base on this study, the antibiotic susceptibility results show that *E. coli* and *Klebsiella* spp. isolated in this study were susceptible to the various antibiotics used while very few were resistant. The relatively low resistance (hence high sensitivity) to the various antibiotics used in this study also confirms that these drugs are still reliable in the treatment of *E. coli* and *Klebsiella pneumonia* infections [12]. It was observed that the multiple antibiotics used for both *E. coli* and *Klebsiella pneumonia* were strongly abused, this could be as a result of wrong prescriptions, misuse and the overuse of drugs. Therefore, it is prudent to screen patients for *E. coli* and *Klebsiella pneumonia* as contact isolation precautions for these patients would go long way in restricting the spread of these organisms and contamination of the environment.

4. CONCLUSION

The results of this study indicate a susceptibility pattern of *E. coli* and *Klebsiella pneumonia* in fecal carriage among patients, which is a major cause of concern. The current data reveals the susceptibility pattern of *E. coli* and *Klebsiella pneumonia* colonization in fecal sample from patients in some hospitals in Port Harcourt. The study will help us to institute authentic resolution, more detailed surveillance studies are required, at the same time this study suggests proper enforcement of antibiotic stewardship, and control measures to contain the spread of *E. coli* and *Klebsiella pneumonia* infections.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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