Original Research Article

Antimicrobial Susceptibility Pattern of *Klebsiella pneumonia* and *Escherichia coli* isolated from Stool Samples of Patients in Some Hospitals in Port Harcourt, Rivers State, Nigeria.

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 some hospitals in Port Harcourt, Rivers State, Nigeria. A total of 114 stool samples were collected from patients and 39 out of 114 samples were *E. coli* and *Klebsiella spp.* positive which comprises of 14 males and 25 females with *E. coli* constituting 19 (36.5%) and *Klebsilla spp.* 20 (38.5%). These isolates were subjected to antimicrobial susceptibility test and it was observed that, Cefuroxime and Augmentin were 100% resistance to *E. coli* followed by Cefixime 78.9%, Ceftazidime 68.4%, Nitrofurantoin 42.1%, Meropenem 10.5% and Ofloxacin which has the least frequency of 0%. The result also reveals that *E. coli* were more susceptible to Meropenem with 89.5% followed by Ofloxacin 84.2%, Ciprofloxacin 73.7%, Gentamicin 68.4%, Ceftazidime 26.3%, Cefuroxime and Augmentin showed no sign of susceptibility. On the contrary, Cefuroxime and Augmentin were highly resistance with (100%) to *Klebsiella spp.* followed by Ceftazidime 70% while Meropenem, Gentamicin, and Ofloxaxin shows 10% resistance to *Klebsiella spp.* Also Meropenem were more susceptible to *Klebsiella spp. (*90%) followed by Ofloxacin 85%, Gentamicin 80%, Nitrofurantoin 40 % and Cefuroxime with 0%. Base on the result obtained, the MAR index of > 0.2 shows suggestive area were drugs are indiscriminately used with *E. coli* having (79%) and *Klebsiella spp.* (85%). Probably because, the drugs were not properly prescribed. From the findings, *E. coli* and *Klebsiella pneumonia* isolated were susceptible to the various antibiotics while few were resistant. Therefore, it is then noted that the various antibiotics used can effectively be used in the treatment of *E. coli* and *Klebsiella* borne infections if prescribed properly to the patients infected.

Key words: antimicrobial susceptibility, Klebsiella, E. coli, stool samples, carbapenem- resistant Enterobacteriaceae

1. INTRODUCTION

With an increase in the number of people being exposed to antibiotics, the intestinal microflora faces constant pressure of antibiotic selection, which has resulted in the emergence of multidrug-resistant strains including carbapenem-resistant strains. This may

pose a severe problem as intestinal *Enterobacteriaceae* are most commonly implicated in human infections 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 stool samples for diagnosing common clinical pathogens may often overlook commensal *Enterobacteriaceae* that can harbour resistant phenotypes. Antibiotic overuse and improper sanitation and hygiene in urban slum areas can lead to the rapid spread and large-scale carriage of multi- or pan-drug-resistant isolates in the intestinal microbiota that can be a potential cause of endogenous and exogenous [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.

An antibiogram is an overall profile pf antimicrobial susceptibility testing results of a specific microorganism to a group of antimicrobial agents/drugs [4]. The subject of antimicrobial sensitivity is of enormous public health microbiology as microorganisms have been shown to device strategies through which they resist the effects of many antimicrobial agents. Successful treatment of diseases caused by microorganisms rely heavily on the ability of public health experts to ascertain antimicrobial agents effective on such microbes hence, the need to continuously study the sensitivity patterns of microbes to antimicrobial agents [5].
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, 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 proof very expensive to treat especially *Escherichia coli* and *Klebsiella pneumoniae* which are the most common pathogens associated with drug resistance and can display resistance to multiple antibiotics even to the recent antibiotics [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 associated with limited therapeutic options and increased mortality in patients infected by these strains [7]. These organisms also have the propensity to undergo widespread dissemination through mobile genetic elements [8]. Enteric strains possessing these carbapenemases have shown remarkable success in the form of large- scale geographical dissemination. 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 in antibacterial activity against CRE isolates [6] with susceptibility rates ranging from 66.2 to100%.

This study was carried out to ascertain the Antimicrobial Susceptibility Pattern of *Klebsiella pneumonia* and *Escherichia coli* isolated from Stool Samples of Patients in Some Hospitals in Port Harcourt, Rivers State, Nigeria.

2. METHODOLOGY

2.1 STUDY AREA

This research study was carried out in two hospitals in Port Harcourt, Rivers State, Nigeria. The study was for a period of three months between January, 2019 to March 2019. Faecal samples were collected using sterile containers. 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 pneumonia* and *Escherichia coli*

Klebsiella pneumonia and *Escherichia coli* were Isolated with Eosin methylene blue (EMB) and MacConkey (MA) agar using streak plate method according to [10] and incubated for 18 to 24hrs at 37^{o}C and examined for growth. Characteristics colonies were described and subcultured onto nutrient Agar plates and incubated for another 24hours for pure cultures (for further tests) were then preserved in sterile 10% glycerol at 4^{o}C. Gramstain reaction and biochemical tests Such as indole, catalase, methyl red, sugar fermentation, 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. The meropenem (10µg) used were manufactured by Zesis Pharmaceuticals PVT limited (India).

Using sterile wire loop, 3-5 colonies of the test organisms was 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 McFarland 0.5) prepared immediately before use. If there was no enough growth, the tube was incubated at 37^{o}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^{o}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^{o}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.
Antibiotic susceptibility of these strains was performed according to Kirby Bauer method as per the Clinical Laboratory Standards Institute guidelines.

3. RESULTS AND DISCUSSION

From the Table 2, Cefuroxime and Augmentin were 100% resistance to *E. coli* followed by Cefixime 78.9%, Ceftazidime 68.4%, Nitrofurantoin 42.1%, Meropenem 10.5% and Ofloxacin which has the least frequency of 0%. The result also reveals that *E. coli* were more susceptible to Meropenem with 89.5% followed by Ofloxacin 84.2%, Ciprofloxacin 73.7%, Gentamicin 68.4%, Ceftazidime 26.3%, Cefuroxime and Augmentin showed no sign of susceptibility. On the contrary, Cefuroxime and Augmentin were highly resistance with (100%) to *Klebsiella spp.* followed by Ceftazidime 70% while Meropenem, Gentamicin, and Ofloxaxin shows 10% resistance to *Klebsiella spp.* it was also observed that Meropenem were more susceptible to *Klebsiella spp.* with 90% susceptibility rate, followed by Ofloxacin 85%, Gentamicin 80%, Nitrofurantoin 40 % and Cefuroxime with 0% as shown in Table 3 below.
When the MAR index is > 0.2 is a suggestive area were drugs are indiscriminately abused. From the result obtained in Table 4 the MAR index of *E. coli* (79%) and *Klebsiella spp.* (85%) reveals areas of drug abuse.

Table 1: Number of *E. coli and Klebsiella Pneumonia* isolates and their percentage occurrence by Sex

Gender	No. of isolates	*E. coli*	*Klebsiella Pneumonia*
Male	20	7 (35.0%)	7 (35.0%)
Female	32	12 (37.5%)	13 (40.6%)
Total	52	19 (36.5%)	20 (38.5%)

Table 2: Susceptibility Pattern of *E. coli* to various antibiotics.

Antibiotics	(Conc.)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Ceftazidime	(30µg)	13 (68.4)	1 (5.3)	5 (26.3)
Cefuroxime	(30µg)	19 (100.0)	0 (0.00)	0 (0.00)
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 (0.00)	0 (0.00)
Cefixime	(5µg)	15 (78.9)	1 (5.3)	3 (15.8)
Nitrofurantoin	(300µg)	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 (0.00)	17 (89.5)

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

Antibiotics Susceptible	(Conc.)	Resistant n (%)	Intermediate n (%)	n (%)
Ceftazidime	(30µg)	14 (70.0)	1 (5.0)	5 (25.0)
Cefuroxime	(30µg)	20 (100.0)	0 (0.00)	0 (0.00)
Gentamicin	(10µg)	2 (10.0)	2 (10.0)	16 (80.0)
Ofloxacin	(5µg)	2 (10.0)	1 (5.0)	17 (85.0)
Augmentin	(30µg)	20 (100.0)	0 (0.00)	0 (0.00)
Cefixime	(5µg)	12 (60.0)	2 (10.0)	6 (30.0)
Nitrofurantoin	(300µg)	9 (45.0)	3 (15.0)	8 (40.0)
Ciprofloxacin	(5µg)	4 (20.0)	4 (20.0)	12 (60.0)
Meropenem	(10µg)	2 (10.0)	0 (00.0)	18 (90.0)

Table 4: Mar Index of *E. coli* and *Klebsiella Pneumonia* isolates.

MAR INDEX	*E. coli* (19) (%)	*Klebsiella* (20) *spp.* (%)
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)	0.00 (0.00)
0.5	0.00 (0.00)	0.00 (0.00)
0.6	0.00 (0.00)	5 (25.0)
0.7	0.00 (0.00)	0.00 (0.00)
0.8	0.00 (0.00)	0.00 (0.00)
0.9	8 (42.2)	0.00 (0.00)
1.0	0.00 (0.00)	9 (45.0)

From the result obtained, it was observed that *E. coli* were highly resistance to Cefuroxime and Augmentin but susceptible to Meropenem, Ofloxaxin, Ciprofloxacin and Gentamicine. *Klebsiella* spare susceptible to Gentamicin, meropenem and Ofloxacin while Cifuroxime and Augmentin were highly resistance. 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. Widespread use of antimicrobials, use of invasive devices and prolonged hospital stay are significant factors contributing to the faecal carriage of which acts *E. coli* and *Klebsiella pneumonia* as a reservoir for dissemination within hospitals. The study also helps 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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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