Antibiotic Resistance Trend of Uropathogenic *Klebsiella pneumoniae* in a Hospital in Abuja, North Central Nigeria, 2014-2015

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Abstract: Antibiotic resistance surveillance is essential in the effective response to the global emergence and spread of multidrug resistant bacteria. This study was carried to determine the trend of antibiotic resistance of *Klebsiella pneumoniae* isolated from urine between January, 2014 and December, 2015 in a hospital in Abuja, North Central Nigeria. Urine samples were collected from patients with suspected cases of UTI who were referred to the Microbiology laboratory. The samples were inoculated onto MacConkey agar and incubated for 24 hours at 37 ^{o}C. Isolates with characteristic colonial morphology of *Klebsiella pneumoniae* were further characterized microscopically and biochemically. Antibiotic susceptibility pattern of the isolates was determined using modified Kirby Bauer disc diffusion method. A total of 44 *Klebsiella pneumoniae* consisting of 22 isolates each from 2014 and 2015 were isolated in this study. The isolates were highly resistant to Ampicillin, Trimethoprim-Sulfamethoxazole and Tetracycline. However, none of the isolates (0.00%) was resistant to Ceftriaxone, Netilmicin and Levofloxacin in 2014 and 2015. An increase in resistance rates of the isolates to Ampicillin, Tetracycline, Ofloxacin and Trimethoprim-Sulfamethoxazole was observed between 2014 and 2015 from: 77.27% to 81.18%, 54.55% to 59.09%, 0.00% to 04.55% and 63.64% to 68.18% respectively. Based on the result of this study, Fluroquinolones, Ceftriaxone, Netilmicin and Levofloxacinmay be considered as therapeutic options for empirical treatment of UTIs caused by *Klebsiella pneumoniae*.

Keywords: *Klebsiella pneumoniae,* resistance, antibiotics, urine, trend

INTRODUCTION

Klebsiella pneumoniae is a Gram-negative, facultatively anaerobic, non-motile, encapsulated and non-flagellated bacillus which belongs to the Enterobacteriaceae family (Al-Zalabani *et al.,* 2020). It is an opportunistic pathogen of clinical and epidemiological significance as it causes life threatening diseases such as bacteremia, urinary and respiratory tract infections (Gavriliu *et al.,* 2016).

Ability of *K. pneumoniae* to acquire mobile genetic elementsis important in the dissemination of resistant genes/plasmids and possibility of *K. pneumoniae* becoming resistant to multiple antibiotics. This also serves as a key factor in the evolution of the pathogen towards becoming more virulent phenotypes (Fasciana *et al.,* 2019).

Urinary tract infection (UTI) is a common health problem in both community and nosocomial settings (Ahmed *et al.,* 2019).When left untreated, UTI may result in serious organ complications such as kidney damage, renal scarring and renal failure. *Klebsiella pneumoniae* is one of the most common Gram negative bacteria implicated in UTI (Nguyen, 2004; Momoh *et al.,* 2011).

One of the greatest advancement of the 20th century is antibiotic therapy. It has succeeded in preventing and treating a great number of infectious diseases and premature deaths (Adedeji, 2016). However, inappropriate use of antibiotics has resulted in antibiotic resistance which has become rising dilemma of significant implications on global public health, showcased by increasing levels of morbidity, mortality and strain on health care systems (Friedman *et al.,* 2016).

Treatment of UTI with broad-spectrum antibiotics empirically without prior culture and sensitivity testing can result in the emergence and spread of multidrug resistant strains of pathogenic bacteria which are resistant to most of the antibiotics (Spellberg *et al.,* 2013).Infections with multidrug-resistant gram-negative pathogens impose a significant and increasing burden on both patients and healthcare providers.

A survey conducted by the European Survey of Antibiotic Consumption revealed that multidrug-resistant (MDR) strains of bacteria were responsible for mortality rate of nearly about 25,000 Europeans/year usually due to complications of UTIs (McQuiston *et al.,* 2013).An outbreak of multidrug-resistant (MDR) *K. pneumoniae* in hospitals is attributed to their ability to spread rapidly (Al-Zalabani *et al.,* 2020).

The abuse and misuse of antibiotics has resulted in resistance to antimicrobial agents which are emerging as a worldwide crisis in the treatment of *K. pneumoniae* associated infections (Navon-Venezia *et al.,* 2017). Antibiotic resistance surveillance is important in monitoring changes in antibiotic susceptibility of uropathogens (Ahmed *et al.,* 2019).

Antimicrobial resistance (AMR) is considered a severe threat to public health in worldwide, it results in increasing costs of treatments, treatment failure and mortality by reducing drug efficacy and limiting the available treatment options (ECDC, 2013; WHO, 2014). AMR surveillance is considered an essential component of an effective response to emerging and spreading antibiotic resistance. The results produced from AMR surveillance will constitute a fundamental source of information on the burden and trends of resistance (ECDC, 2013).

The clinical significance of the pathogenic bacteria and its implications on infection control, as well as the emergence of multi drug resistant strains, signify the importance of local surveillance of antibiotic-resistant bacteria (Al-Zalabani *et al.,* 2020).

MATERIALS AND METHODS

Collection of samples

Mid-stream urine (10 mL) samples were collected from patients referred to the Microbiology laboratory of a hospital in Abuja, North Central Nigeria for suspected case of urinary tract infection (UTI) in sterile, dry, wide-necked, leak-proof containers. The samples were collected between January 2014 and December 2015.

Isolation and Characterization

Loopful of the urine samples were inoculated on MacConkey agar by streaking and incubated for 34 hours at 37ºC. Isolates with characteristic colonial morphology of *Klebsiella pneumoniae* on MacConkey agar (pink mucoid colonies)were further characterized microscopically by Gram staining and biochemically using the following biochemical tests: Indole test, Methyl Red - Voges-Proskauer test, Citrate utilisation test, Sugar fermentation test, Urease test and Motility test as described by Cowan and Steel (2003).

Antibiotic Susceptibility Test

Antibiotic susceptibility pattern of the isolates was determined by modified Kirby-Bauer disc diffusion technique as described by Acharya (2013).Antibiotics susceptibility test was carried out on Mueller Hinton agar using the following antibiotic discs: Ampicillin (10 µg), Ceftriaxone (30µg), Cefuroxime (30µg), Gentamicin (10µg), Netilmicin (30 µg), Azithromycin (15 µg), Tetracycline (30µg), Levofloxacin (5 µg), Ofloxacin (5 µg),Nitrofurantoin (300 µg) and Trimethoprim-Sulfamethoxazole (1.25/23.75 µg). The diameters of the zone of inhibition were measured with the aid of a ruler to the nearest mm. Using the published CLSI guidelines, the susceptibility or resistance of the isolates to each of the antibiotic tested was determined (CLSI, 2014; CLSI, 2015).

Statistical analysis

The differences in resistance rates were analyzed statistically for significant changes during the one year period using Chi square test. P value was considered significant for a value less than or equal to 0.05.

RESULTS

Figure 1 show the antibiotic resistance rates of *Klebsiella pneumoniae* isolated from urine in 2014 and 2015. The isolates were highly resistant to Ampicillin (77.27% in 2014 and 81.18% in 2015), Trimethoprim-Sulfamethoxazole (63.64% in 2014 and 68.18% in 2015) and Tetracycline (54.55% in 2014 and 59.09% in 2015).

Moderate resistance rate was observed to Nitrofurantoin (31.82% in 2014 and 04.55% in 2015) and Gentamicin (31.82% in 2014 and 18.18% in 2015). Low resistance rates were observed to Cefuroxime (09.09% in 2014 and 04.55% in 2015), Azithromycin (09.09% in 2014 and 04.55% in 2015) and Ofloxacin (0.00% in 2014 and 04.55% in 2015). None of the isolates (0.00%) were resistant to Ceftriaxone, Netilmicin and Levofloxacin in 204 and 2015.

The number (percentage) resistance of *Klebsiella pneumoniae* isolated from urine and resistance evolution of the isolates in 2014 and 2015 is presented in Table 1. Increase in resistance rates were observed to Ampicillin (from 77.27% to 81.18%), Tetracycline (from 54.55% to 59.09%), Ofloxacin (from 0.00% to 04.55%) and Trimethoprim-Sulfamethoxazole (from 63.64% to 68.18%) between 2014 and 2015 respectively. However, decrease in resistance rates were observed to Cefuroxime (from 09.09% to 04.55%), Gentamicin (from 31.82% to 18.18%), Azithromycin (from 09.09% to 04.55%) and Nitrofurantoin (from 31.82% to 4.55%) between 2014 and 2015. No change in resistance rate was observed to Ceftriaxone, Netilmicin and Levofloxacin. The differences observed in resistance rates in 2014 and 2015 were not statistically significant ($p > 0.05$) except for Nitrofurantoin ($p = 0.05$).

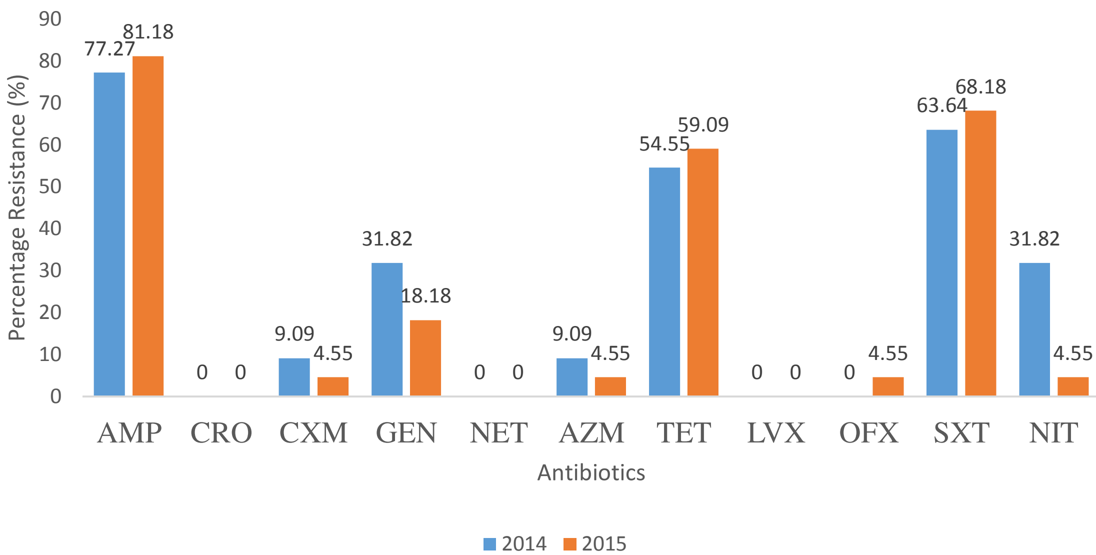

Figure 1: Antibiotic resistance rates of *Klebsiella pneumoniae* isolated from urine in 2014 and 2015

Key: AMP = Ampicillin, CRO = Ceftriaxone, CXM = Cefuroxime, GEN = Gentamicin, NET = Netilmicin, AZM = Azithromycin, TET = Tetracycline, LVX = Levofloxacin, OFX = Ofloxacin, SXT = Trimethoprim-sulfamethoxazole, NIT = Nitrofurantoin

Table 1: Number (percentage) and resistance evolution of *Klebsiella pneumoniae* between 2014 and 2015

Antibiotic class	Antibiotic	No. (%) of isolates resistant 2014 (n=22)	2015 (n=22)	p-value
Penicillin	Ampicillin	17 (77.27)	18 (81.18)	0.71
Cephalosporin	Ceftriaxone	00 (00.00)	00 (00.00)	-
	Cefuroxime	02 (09.09)	01 (04.55)	0.55
Aminoglycoside	Gentamicin	07 (31.82)	04 (18.18)	0.49
	Netilmicin	00 (00.00)	00 (00.00)	-
Microlide	Azithromycin	02 (09.09)	01 (04.55)	0.55
Tetracycline	Tetracycline	12 (54.55)	13 (59.09)	0.76
Fluoroquinolone	Levofloxacin	00 (00.00)	00 (00.00)	-
	Ofloxacin	00 (00.00)	01 (04.55)	0.31
Folate pathway antagonist	Trimethoprim-Sulfamethoxazole	14 (63.64)	15 (68.18)	0.75
Nitrofurans	Nitrofurantoin	07 (31.82)	01 (04.55)	0.05*

DISCUSSION

The emergence and spread of MDR bacteria in hospital environment is increasing worldwide. Antimicrobial resistance is one of the greatest challenges worldwide and arises due to the overuse and misuse of antibiotics in modern medicine and animal husbandry, which significantly threatens public health (Mhondoro *et al*., 2019).

In this study, high rates of resistance were found in commonly prescribed and used antibiotics namely Ampicillin, Tetracycline and Trimethoprim-Sulfamethoxazole. This result is in line with the report of Ahmed *et al.* (2019) and Al-Zalabani *et al*. (2020) in Saudi Arabia where highest resistance rates were observed to Ampicillin, Tetracycline and Trimethoprim-Sulfamethoxazole. The high resistance rates observed may be due to the fact that they are easily accessible over the often without prescription.

Low resistance rates were observedto Aminoglycosides (Netilmicin and Gentamicin) and Fluoroquinolones (Levofloxacin and Ofloxacin). These low resistance rates might be due to the fact that these antibiotics are not easily available and/or relatively expensive compared to others in the study area. This is in consonant with the low resistance rates observed by Ahmed *et al.* (2019)and Al-Zalabani *et al.*(2020)both in Saudi Arabia to Aminoglycosides and Fluoroquinolones.

Low resistance rates were found in cephalosporins tested, with Ceftriaxone (a third generation cephalosporin) having lower resistant rate compared to Cefuroxime (a second generation cephalosporin). This indicates that most of the isolates screened were not harbouring genes that codes for cephalosporin hydrolyzing enzymes such as ESBL and AmpC (Braykov *et al.,* 2013).

A decrease in resistance rates of *Klebsiella pneumoniae* was observed to Gentamicin and Cefuroxime between 2014 and 2015. This decrease in resistance rates of the isolates to Gentamicin and Cefuroxime over time is in agreement to the report of Hu *et al.*(2016) in China who reported a decrease in resistance rate of *Klebsiella pneumoniae* to Gentamicin and Cefuroxime between 2008 and 2014.However, the resistance rate of the isolates to Gentamicin in 2014 observed in this study (31.82%) is higher than 24.6% reported by Hu *et al.* (2016).Increase in resistance rate to Trimethoprim-Sulfamethoxazole was observed in this study between 2014 and 2015.

This might be due to the frequent use of this antibiotic as prophylaxis. This is in contrast to the fluctuating Trimethoprim-Sulfamethoxazole resistance rates of *K. pneumoniae* between 2005 and 2014 reported by Hu *et al.* (2016).

None apparent changes in the resistance rates to Ceftriaxone, Netilmicin and Levofloxacin observed as well as the decrease in resistance rates to Cefuroxime, Gentamicin, Azithromycin and Nitrofurantoin suggest these antibiotics are still a valuable alternative for treatment of UTIs. Therefore, these drugs could be considered as therapeutic options for empirical treatment of UTIs caused by *K. pneumoniae* in this study subjects.

Increased rate of ofloxacin resistant isolates between 2014 and 2015 observed in this study is in line with the report of Guyomard-Rabenirina *et al.* (2016) who also reported an increased in Ofloxacin resistance rate in Guadeloupe, France.

Low rates of Fluoroquinolones resistant isolates observed in this study lend credence to the suggested use of Fluoroquinolones as reasonable empiric treatment for UTI by Al-Zahrani*et al.*(2019). However, the increasing use of Fluoroquinolones raises concerns regarding the possibility of increasing resistance rates. This is demonstrated by the increased rate of Ofloxacin resistance between 2014 and 2015 in this study. Hence the announcement by US Food and Drug Administration in 2016 that the package insert of fluoroquinolones will be updated to include a warning stating that fluoroquinolones should not be used for routine respiratory tract infections or uncomplicated UTIs unless there is no suitable alternative agent (US FDA, 2016).

CONCLUSION

The highest antibiotic resistance rate of *K. pneumoniae* was observed to Ampicillin, while the least resistance rates were observed to Ceftriaxone, Levofloxacin, Netilmicin and Ofloxacin. Increased antibiotic resistance rates of *K. pneumoniae* between 2014 and 2015 were observed in Ampicillin, Tetracycline, Ofloxacin and Trimethoprim-Sulfamethoxazole.

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