***Original Research Article***

**Evaluation of Occurrence and Antibiotic Resistance Patterns of *Klebsiella pneumonia* from urine of students in tertiary institution**

**ABSTRACT**

*Klebsiella pneumoniae* is a common bacterium responsible for many cases of urinary tract infections (UTIs). The growing resistance of this organism to various antibiotics is reducing the number of effective treatment choices. The aim of this study is to ascertain the occurrence and antibiotic resistance patterns of *Klebsiella pneumoniae* isolated from urine of students of Applied Microbiology, Enugu State University of Science and Technology. A total of 67 urine samples were collected aseptically from apparently heathy students. A loopful of each urine sample was inoculated on MacConkey agar for a significant bacterial growth. The isolates were identified using standard microbiological procedures. Results showed mean bacterial load of 1.7×104  cfu/ml from males and 2.1×104 cfu/ml from females. Out of 67 urine samples collected from students, 17 (25.37%) yielded positive growth of *Klebsiella pneumoniae*. The male students had 6 (18.75%) from 32 urine samples while female students had 11 (31.43%) from 35 urine samples. There was no significant difference (p˃0.05) in the prevalence rates among the males and females. Test organism had varying degrees of resistance on the antibiotics used. *Klebsiella pneumoniae* from male*s* had resistance to the following antibiotics: amoxacillin at 83.3%; streptomycin and ofloxacin at 50% respectively; cotrimoxazole, augmentin and pefloxacin at 66.67% respectively; gentamycin at 83.3% while *Klebsiella pneumoniae* from females had resistance on amoxacillin at 81.82%;streptomycin at 54.53%; sparfloxacin at 72.73%; ciprofloxacin at 63.64%. Generally, the females had more resistance to the antibiotics than the males. Multidrug resistance (MDR) index ranged from 0.1 to 0.5 for males and 0.2 to 0.9 for females. The high prevalence of *Klebsiella pneumoniae* and MDR index among the students in this study was a threat to their health and indicative of poor personal hygiene. Therefore, public enlightenment programmes should be carried out to educate them on the prevention of urinary tract infections.

**Key Words:** Antimicrobial resistance, Multiple antibiotic resistant index, Urine, Urinary tract infections (UTIs), *Klebsiella pneumoniae*

1. **INTRODUCTION**

Antimicrobial resistance (AMR) among microorganisms is a public health problem especially among underdeveloped countries. This has affected the treatment of different diseases to a great extent. Antibiotic-resistant bacterial infections, particularly those caused by multidrug-resistant (MDR) strains, can result in major health issues such as extended hospital stays, unsuccessful treatments, and even death (Ayandele *et al*., 2020). The indiscriminate use of antimicrobial drugs in agriculture, aquaculture, human and animal medicine is one of the main causes of bacterial resistance (Khan *et al*., 2015). Multiple AMR occurs as a result of the presence of plasmids containing one or more resistant genes, each of which encodes a single phenotype. These genes can be transferred to other bacteria of the same species or different ones (Di Tella *et al*., 2019). Bacteria develop resistance to antibiotics through a variety of mechanisms, some of which are dominant and identifiable (Ayandele *et al*., 2020).

 The multiple antibiotic resistance (MAR) index offers a practical, accurate and affordable way to identify the source of antibiotic-resistant bacteria. 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 (Khan *et al*., 2015). An MAR index above 0.2 suggests that the bacterial isolate comes from a setting where antibiotics are frequently or extensively used (Mir *et al*., 2022). The study of the multiple antibiotic resistance index of *Klebsiella pneumoniae* is of paramount importance given the increasing threat of antibiotic resistant infections. Investigating antibiotic resistance can clarify its extent, guide medical treatment and shape policies to combat its spread.

Urine is a liquid by-product of metabolism in humans and in many other animals. Urine flows from the kidneys through the ureters to the urinary bladder. Urination results in urine being excreted from the body through the urethra (Zalmanovici *et al.,* 2010). Cellular metabolism generates many by-products that are rich in nitrogen and must be cleared from the bloodstream, such as urea, uric acid, and creatinine. These by-products are expelled from the body during urination, which is the primary method for excreting water-soluble chemicals from the body (Onah *et al.,* 2006). A urinalysis can detect nitrogenous wastes of the mammalian body. Urine plays an important role in the earth's nitrogen cycle. In balanced ecosystems, urine fertilizes the soil and thus helps plants to grow. Therefore, urine can be used as a fertilizer. Some animals use it to mark their territories.

Urinary Tract Infections (UTIs) are infections caused by the presence and replication of microorganisms in the urinary tract. It is the single most common bacterial infection of mankind (Morgan and McKenzie, 1993; Ebie *et al*., 2001). Females are believed to be more affected than males except at the extremes of life (Ebie *et al*., 2001; Kolawole *et al*., 2009). This is because bacteria can reach the bladder more easily in women, partially due to the short and wider female urethra, and its proximity to the anus. Available scientific information indicates that bacteria easily travel up to the urethra from the rectum and thereby causing infection (Ebie *et al*., 2001; Kolawole *et al*., 2009). Urinary tract infections (UTIs) are among the most common bacterial infections in adults and may involve the lower or upper urinary tract or both (Turay *et al.,* 2014). Asymptomatic bacteriuria refers to considerable bacteriuria in a woman with no symptoms (Onah *et al.,* 2006). When the infection is limited to the lower urinary tract and occurs with symptoms of dysuria and frequent and urgent urination and, occasionally, suprapubic tenderness, it is termed cystitis*.* Acute pyelonephritis is defined as infection of the renal paren-chyma and pelvicaliceal system accompanied by significant bacteriuria, usually occurring with fever and flank pain (Almehdawi *et al.,* 2016).

*Klebsiella pneumoniae* is a Gram-negative, rod shaped, non-motile, facultative anaerobic bacteria. *Klebsiella pneumoniae* is an emerging threat to human health as it causes both endemic and epidemic infections. Most hospital acquired infections caused by *Klebsiella pneumoniae* cause a challenge in their treatment because of the development of MDR strains. The resistance of *Klebsiella pneumoniae* strains to different generations of cephalosporins, especially the third generation, was first reported in 1981; since then, these bacteria have become more resistant to several antibiotics (Ayandele *et al*., 2020). This may be due to poor healthcare infrastructure and inappropriate antibiotic use. Some studies have highlighted the escalating antibiotic resistance in *Klebsiella pneumoniae* strains isolated from clinical specimens in Nigeria. (Ayandele *et al*., 2020; Osundiya *et al*.,2013). Many routinely used antibiotics, including β-lactams (including extended-spectrum cephalosporins and carbapenems), Chew *et al*., 2017; Wang *et al*., 2022), fluoroquinolones (Geetha *et al*., 2020) and aminoglycosides, (Andrade *et al*., 2014) have seen a significant rise in resistance. Not many research works have been documented on the MAR index of *Klebsiella pneumoniae* in Nigeria especially in this study area. Hence, there is the need for more research to be done in this area. The aim of this study is to ascertain the occurrence and antibiotic resistance patterns of *Klebsiella pneumoniae* isolated from urine of students of tertiary institution.

**2. MATERIALS AND METHODS**

* 1. **Study Design**

Experimental research design was used in this research work.

* 1. **Study Population**

A total of 67 students in the Department of Applied Microbiology of the University were used as the study population.

**2.3 Study Area**

The study was conducted at the Department of Applied Microbiology of Enugu State University of Science and Technology. The University is located on latitude 6.49460N and longitude 7.49600 E.

**2.4 Sample Collection**

Mid-stream urine samples were collected from sixty-seven (67) students (both male and female) of Department of Applied Microbiology in Enugu State University of Science and Technology, Agbani campus who willingly submitted for analysis. The samples were collected aseptically in sterile urine containers. The samples were immediately labelled and taken to the laboratory for bacteriological analysis.

**2.5 Isolation and Characterization of isolates**

 A loopful of the urine samples were inoculated into sterile duplicate MacConkey agar plates and were incubated at 370C for 24 hours. The pure cultures of the isolates were subcultured on sterile nutrient agar plates. The isolates were identified using standard microbiological methods and biochemical tests according to Cheesbrough (2006). The isolates were stored on nutrient agar slants at 40C for further analysis. The biochemical tests are catalase, coagulase, oxidase, indole, citrate utilization and sugar fermentation tests.

**2.5.1 Indole Test**

Sterile test tubes containing 5 ml of tryptophan broth were set on a test tube rack, the tubes were inoculated aseptically and the bacteria growth added into it. The tubes were incubated at 37 ºC for 24 h. After 24 h, 0.5 ml of kovac’s reagent was added to it and allowed to stand for 5 minutes, formation of pink or red colour ring in the reagent layer on the medium (within 10 seconds) indicates positive result. Negative result shows no formation of pink or red colour ring.

**2.5.2 Citrate utilization Test**

Simon citrate agar was prepared and sterilized into a test tube and slanted. It was allowed to solidify before organism was inoculated on the surface of the solidified Simon citrate agar in the test tube. It was covered with cotton wool and incubated at room temperature for 24 hours. For positive result, there will be visible growth and the medium will be blue while the negative result showed no visible growth and no colour change.

**2.5.2 Catalase Test**

Catalase test was done using a test tube; A clean test tube was placed on the rack, 1ml of hydrogen peroxide solution was poured into the test tube; Using a sterile glass rod, bacteria growth were picked from an agar plate and immerse it into the hydrogen peroxide solution. Presence of effervescence indicated catalase positive reaction whereas negative reaction showed no effervescence.

**2.5.3 Oxidase Test**

This was done using filter paper. A piece of filter paper was moistened with few drops of oxidase reagent. A sterile wire loop was used to pick a colony of the organism from the agar plate and was smeared onto the moistened filter paper. It was observed for a colour change within 10 – 30 seconds. Dark purple or blue colour developed within 10 seconds indicated a positive result while no colour change indicated a negative result.

**2.5.4 Sugar Fermentations**

A 10 ml of peptone water was introduced into 5 sterile test tubes respectively. Three (3) drops of methyl red was added into each of the test tubes, then Durham’s tubes were inserted in an inverted position into each of the tubes and sealed with foil before sterilization in an autoclave at 121 ºC for 10 minutes. One gram (1g) of respective carbohydrates: glucose, lactose, fructose, sucrose and mannitol, were added into 100 ml of sterile distill water and sterilized using membrane filter. A total of 1 ml of each of sterile sugar was added into each of the sterilized test tubes that contained the peptone water. Thereafter, the cultured organisms were inoculated into each of the tubes respectively. They were then incubated at 37 ºC for 24 h. Positive result indicates yellow colour while gas production were seen in the Durham’s tube.

**2.6 Antimicrobial Susceptibility Testing**

An antibiotic susceptibility test was performed using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar plates. Bacterial inoculum was prepared to match 0.5 McFarland turbidity standard for each isolate and a loop full of the urine sample was swabbed on already prepared Mueller Hinton agar plates. Ten commercially made antibiotics were placed on the duplicate Mueller Hinton agar plates which were then incubated at 370C for 24 hours. The antibiotics used include: Cotrimoxazole (30 μg), Chloranphenicol (30 μg), Sparfloxacin (10 μg), Ciprofloxacin (30 μg), Amoxacillin (30 μg), Augmentin (30 μg), Gentamycin (30 μg), Pefloxacin (30 μg), Ofloxacin (10 μg), Streptomycin (30 μg). Zones of inhibition diameter were measured in milimeter (mm) and compared with Clinical Laboratory Standard Institute to determine their resistance patterns (CLSI, 2014).

**2.7 Determination of MAR Index**

Determination of MAR index was carried out as described by Khan *et al*.(2015) in which the number of antibiotics an isolate is resistant to (a) is divided by the total number of the antibiotics used in the study (b). The calculating formula is:

MAR index = a/b where a = number of antibiotics an isolate is resistant to and b= total number of the antibiotics used in the study.

**2.8 Statistical Analysis**

The data from this study were analyzed using SPSS version 21.0 and ANOVA.

**3. RESULTS**

**3.1 Percentage (%) Occurrence of *Klebsiella pneumonia*e in urine of some students of tertiary institution**

The percentage (%) occurrence *Klebsiella pneumoniae* in urine of some students were screened and it was found out that out of 67 urine samples collected, 17 (25.37%) of the students harbor the organism. The result is shown in Table 1.

**Table 1: Percentage Occurrence of *Klebsiella pneumoniae* in urine of some students of tertiary institution**

|  |  |  |  |
| --- | --- | --- | --- |
| Sex | Number of urine samples collected | Number positive (%)  | Number negative (%) |
| Male | 32 | 6 (18.75%) | 26 (81.25%) |
| Female | 35 | 11 (31.43%) | 24 (68.57%) |
| Total | 67 | 17 (25.37%) | 50 (74.63%) |

**3.2 Determination of mean bacterial load (cfu/ml) of urine samples screened**

The mean bacterial load of some of the urine samples from the students were determined. The male students had 1.7×104 cfu/ml while female students had 2.1×104 cfu/ml. The result is shown in Table 2.

**Table 2: Mean bacterial load (cfu/ml) of urine samples screened**

|  |  |
| --- | --- |
| Sex | Mean bacterial load (cfu/ml) |
| Male | 1.7×104 |
| Female | 2.1×104 |

**Table 3: Biochemical identification of *Klebsiella* Isolates from Urine Samples**

|  |  |
| --- | --- |
| **Biochemical tests** | ***Klebsiella pneumonia*** |
| Gram reaction | Gram-negative rod |
| Indole test | Negative |
| Oxidase test | Negative |
| Citrate test | Positive |
| Catalase | Positive |
| **Sugar fermentation** |  |
| Glucose | Positive |
| Fructose | Positive |
| Galactose | Positive |
| Maltose | Positive |
| Sucrose | Positive |

**3.3: Antibiotic Resistance profiles of *Klebsiella pneumoniae* among students of tertiary institution**

The antibiotic resistance profiles of *Klebsiella pneumoniae* were determined. It was found out that the test organism had varying degrees of resistance on the antibiotics used. Females had 66.67% of resistance on chloramphenicol while males had 52.94% on pefloxacin. Generally, the females had more resistance to the antibiotics than the males. The results are shown in Table 4.

**Table 4: Antibiotic Resistance profiles of *Klebsiella pneumonia*e among students of tertiary institution**

|  |  |  |
| --- | --- | --- |
| Antibiotics | Males (n=6) (%)  | Females (n=11) (%) |
| Amoxacillin | 5 (83.30) | 9 (81.82) |
| Streptomycin | 3 (50.0) | 6 (54.55) |
| Cotrimoxazole | 4 (66.67) | 4 (36.3673) |
| Chlorampenicol | 3 (50.0) | 3 (27.27) |
| Sparfloxacin | 1 (16.67) | 8(72.73) |
| Ciprofloxacin | 2 (33.33) | 7 (63.64) |
| Augmentin | 4 (66.67) | 4 (36.36) |
| Gentamycin | 5 (83.30) | 5 (45.46) |
| Pefloxacin | 4 (66.67) | 3 (27.27) |
| Ofloxacin | 3 (50.0) | 2 (18.18) |

**3.4 Multidrug Resistance Index of *Klebsiella pneumoniae* among students of tertiary institution**

The multidrug resistance index of the test organism was determined. It was observed that both males and females had high multidrug resistance index ranging from 0.1 to 0.5 for males and 0.2 to 0.9 for females. The result is shown in Table 5.

**Table 5: Multidrug Resistance Index of *Klebsiella pneumoniae* among students of tertiary institution**

|  |  |  |
| --- | --- | --- |
| Antibiotics | Males  | Females  |
| Amoxacillin | 0.5 | 0.9 |
| Streptomycin | 0.3 | 0.6 |
| Cotrimoxazole | 0.4 | 0.4 |
| Chlorampenicol | 0.3 | 0.3 |
| Sparfloxacin | 0.1 | 0.8 |
| Ciprofloxacin | 0.2 | 0.7 |
| Augmentin | 0.4 | 0.4 |
| Gentamycin | 0.5 | 0.5 |
| Pefloxacin | 0.4 | 0.3 |
| Ofloxacin | 0.3 | 0.2 |

**4. DISCUSSION**

Multidrug resistance among uropathogens have been a public health problem worldwide. The isolation of these pathogens from individual especially students is alarming. In this present study, out of 67 urine samples collected from students, 17 (25.37%) yielded positive growth of *Klebsiella pneumoniae* (Table 1). The male students had 6 (18.75%) from 32 urine samples while female students had 11 (31.43%) from 35 urine samples (Table 1). Here, there was no significant difference (p˃0.05) in the prevalence rates among the males and females. This prevalence rate is higher than the result from the work of Barwa *et al*.( 2022) who had 0.7% of *Klebsiella pneumoniae* from students. From this study, female students had higher occurrence of the organism than the males. This is not in agreement with the work of Barwa *et al*.(2022) who had 1(0.1%) of *Klebsiella pneumoniae*. This study is in tandem with the work of Turay *et al*. (2014) who had 18.2% of *Klebsiella pneumoniae* from pregnant women. The reason for this high prevalence rate of this isolate among female students could be attributed to the fact that females have shorter urethra which alloiws bacteria from the urethral meatus and the perineum to gain access into the bladder (Amali *et al*., 2009; Bishop and Shehn, 2016). It can also be due to prevailling poor housing and drainage systems in the school environment as well as lack of proper personal environmental hygiene (Turay *et al*., 2014), and indiscriminate sexual intercourse among university students. The high rate of the isolate could be as a result of improper use of the toilet which could result in infections as urine is ejected with force and creates great splashes which could re-introduce pathogenic organisms from the environment into the urinary opening (Barwa *et al*., 2022). The study revealed mean bacterial load of 1.7 x104cfu/ml from males and 2.1 x104cfu/ml from females (Table 2). High bacterial load was observed in the urine samples from female students in ESUT. The high bacterial load shows that urine is a great reservoir for diverse microorganisms especially bacteria, some of which can be potential pathogens of public health importance. The present study revealed high multidrug resistance to some antibiotics tested among males and females in the Department of Applied Microbiology. *Klebsiella pneumoniae* from male*s* had resistance to the following antibiotics: amoxacillin at 83.3%; streptomycin and ofloxacin at 50% respectively; cotrimoxazole, augmentin and pefloxacin at 66.67% respectively; gentamycin at 83.3% while *Klebsiella pneumoniae* from females had resistance on amoxacillin at 81.82%;streptomycin at 54.53%; sparfloxacin at 72.73%; ciprofloxacin at 63.64% (Table 4). It was observed from the study that *Klebsiella pneumoniae* is resistant to more than two classes of antibiotics used in the study: β-lactam (amoxacillin:male 83.3%;female 81.82%); quinolones (ofloxacin: male 50%; female 18.18%); aminoglycosides( gentamycin: male 83.3%; female 45.46%). The study is in agreement with the work of Barwa *et al*. (2022) who reported resistance of *Klebsiella pneumoniae* to cotrimoxazole and augmentin; susceptible to ciprofloxacin and sparfloxacin. This study is also in agreement with the work of Ogefere and Idoko (2024) who reported 61% of resistance to gentamycin. Also this study is in line with the work of Ayandele *et al*. (2020) which had resistance rate of ofloxacin at 80% and gentamycin at 70%. The differences in values of resistance could be as a result of differences in strains and geographical locations (Sun *et al*., 2022) The study is in contrast with the work of Osundiya *et al*.(2013) who had sensitivity to ofloxacin at 91%. This high multidrug resistance observed in this study could be traced to *Klebsiella pneumoniae* harboring plasmids and transposomal genes which can mediate antibacterial resistance (Ogefere and Idoko, 2024). The present study showed the multidrug multidrug resistance index of *Klebsiella pneumoniae* among the male and female students (Table 5). The value of the multidrug resistance index were consistent ranging from 0.1 to 0.9, though the females had higher multidrug resistant index. This study is in line with the work of Ogefere and Idoko (2024) who had multidrug resistant index ranging from 0.42 to 1.00.This MDR index observed from this study could be as a result of the students using drugs extensively or indiscriminately without doctor’s prescription. Calculating the MDR index of these bacterial isolates is crucial for understanding their resistance patterns and assessing their potential impact on public health.

**5. CONCLUSION**

*Klebsiella pneumoniae* was isolated from urine of students of Applied Microbiology with high prevalence rate. It is crucial to maintain good personal hygiene to reduce urinary tract infection caused by this organism. Also this study recorded high MDR index. To prevent antibiotic resistance, it is essential to carefully choose the right antibiotic and use it responsibly, following proper dosage and administration guidelines.

**ETHICAL APPROVAL**

Ethical approval was gotten from the ethical committee of the Faculty of Biological Sciences of the University.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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