

Original article

Antibiotic sensitivity profile of bacteria isolated from urinary catheters in urinary tract infections' patients

Mohammed Bashir ^{*1}, H. Isa ¹, K. I. Chimbekujwo ¹, N. A. Kachalla ¹, A. B. Umar ², M. Bilyaminu ²

1- Department of Microbiology, Modibbo Adama University of Technology, Yola, Nigeria.

2- Department of Science Laboratory Technology, Modibbo Adama University of Technology, Yola, Nigeria.

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ABSTRACT

Background: Urinary catheterization is a common procedure found in hospitals, and is even more common in intensive care units. There are risk factors associated with urinary tract infection (UTI), of which the use of a urinary catheter accounts for more than 80% of all health care associated UTIs. This study was conducted to determine the antibiotic susceptibility profile of bacteria associated with urinary catheters from UTI patients. **Methods:** Urine samples were collected aseptically from a removed catheter within 24 hours of insertion and cultured on blood agar and Cysteine Lactose Electrolyte Deficient Agar (CLED) and identified. Antibiotic susceptibility test was conducted using disk diffusion method on Mueller Hinton agar. **Results:** A total of 47 isolates were obtained, *E. coli* 15(31.92%), *P. aeruginosa* 4(8.51%), *P. mirabilis* 5(10.64%), *P. vulgaris* 5(10.64%), *K. pneumonia* 3(6.38%), *S. aureus* 11(23.40%), *S. saprophyticus* 4(8.51%) and showed various degrees of resistance and susceptibility to various antibiotics. *E. coli* was 73.3% and 66.7% resistance to gentamycin and cefotaxime respectively. *P. aeruginosa* showed 100% and 50% resistance to Nitrofurantoin and cefotaxime respectively, *P. mirabilis* showed 100% resistance to cefotaxime and 60% to gentamycin, nalidixic acid and tetracycline. *P. vulgaris* was also 100% resistance to gentamycin, ampicillin, cefotaxime and 80% to tetracycline. *K. Pneumonia* showed 100% resistance to tetracycline, ampicillin and norfloxacin. *S. Saprophyticus* showed 100% resistance to only teicoplanin. However, all the isolates were 100% susceptible to at least one antibiotic. **Conclusion:** The bacterial pathogens associated with infection in urinary catheters in this study respond to different antibiotics at different degrees ranging from sensitive to resistant.

Introduction

Urinary tract infection (UTI) is defined as a condition in which the urinary tract is infected with a pathogen causing inflammation which is a common, distressing and occasionally life threatening condition [1]. Urinary tract infection affects people of all ages and gender. Females are more susceptible to UTIs compared to males [2].

Some of the key factors predisposing to UTIs have been attributed to poor personal hygiene and urinary tract abnormalities [3]. The use of a urinary catheter is the biggest risk factor for UTI globally, accounting for more than 80% of all nosocomial UTIs [4]. The burden of UTIs on both the individuals and the society has several factors and may likely increase the resistant to antibiotics.

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* Corresponding author: Mohammed Bashir

E-mail address: mbashir@mautech.edu.ng

Effective utilization of prophylaxis can result to ease the burden of UTIs on both patients and the society [5].

The presence of the indwelling catheter inside the bladder makes the patient much more susceptible to infections. Normally, the urinary tract is kept sterile by the flow of urine because the urine always flushes out bacteria. In addition to that, the urethral sphincter helps to keep the bladder closed and prevents the reflux of contaminated urine back into the bladder [6]. However, the catheter system bypasses the urethral sphincter meaning that bacteria are able to gain access to the urinary tract and bladder, either intra- or extra-luminally [6]. If there are any breaks in the closed catheter system, bacteria will enter intra-luminally [2]. Extra-luminal bacterial contamination often occurs during the insertion process, spread from health-care workers' hands, or from the patient's normal flora [6].

Treatment of UTI cases are often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens [7,8]. However, the choice of antibiotic depends on the type of bacteria, level of infection and symptoms. Urinary tract infections are often treated with different broad-spectrum antibiotics when one with a narrow spectrum of activity may be effective in killing the organisms, because of concerns about antibiotic resistant. The threat of antimicrobial resistance has extended from the hospital setting to the community setting. The trend in increased antimicrobial resistance among bacterial pathogens that cause UTIs severely limits the choice of effective antimicrobial agents [9]. This study was conducted to determine the sensitivity profile of bacteria that are associated with catheters from individuals with UTIs in Dawwa'u and Meddy clinics Jimeta, Adamawa state.

Materials and Methods

Description of the study area

Jimeta is a town in Adamawa state; in the north eastern Nigeria, it lies on the south bank of the Benue River, and on the highway between Zing and Girei. Jimeta has a population of 73,080 in 1991. It has elevation of 135m and it lies along the Benue River. Jimeta is located at latitude $9^{\circ} 16' 60''$ N and longitude $12^{\circ} 28' 00''$ E [10].

This study was carried out within a period of 6 months (between September, 2018 to February, 2019) in Jimeta, Yola Adamawa state Nigeria. A total of 50 samples were collected using

convenience sampling as described by **Hamed** [11] from catheterized patients in Dawwa'u and Meddy clinics within Jimeta metropolis. Urine samples were collected aseptically in the morning between 8 to 10am from a catheter that was removed within 24hrs of insertion, the samples were collected in a sterile container labeled and transported at ambient temperature [12] to microbiology laboratory Modibbo Adama University of technology, Yola within one hour of collection.

Isolation and identification of bacteria from urine samples

Direct (macroscopic) examination of the urine such as the colour and appearance of the urine was carried out. The urine samples were inoculated using streaked plate method onto Cystine Lactose Electrolyte Deficient Agar (CLED) and blood agar. The plates were incubated at 37°C for 24hrs by using standard laboratory techniques. Identification of isolates was done by examining the morphological characteristics of the colonies, Gram's staining and certain biochemical tests such as catalase, coagulase, methyl red, indole, citrate utilization, urease, oxidase, novobiocin, nitrate reduction test [13].

Antibiotic susceptibility tests

The isolated bacteria were tested on Mueller – Hinton agar (Oxoid, UK) using disk diffusion method according to clinical and laboratory standard institute (CLSI) [13] guidelines. The suspension of the organisms was prepared by taking 5 colonies of 24hrs culture and transfer to 5mL of trypticase soy broth and incubated at 30°C and the turbidity was adjusted to 0.5 McFarland standards [14]. The identified bacteria were tested against norfloxacin (10mg), cephalothin (30mg), ceftriaxone (30mg), tetracycline (30mg), nitrofrontoin (10mg), chloramphenicol (10mg), cephalothin (50mg), Nalxidic acid (30mg), cefotaxime (30mg), ampicillin (10mg), penicillin (30mg), erythromycin (15mg), gentamicin (10mg), vancomycin (30mg), ciproflaxacin (30mg), teicoplanin (30mg) (Liofilchem). The plates were incubated at 37°C for 24hrs and the diameter of each inhibition zone was measured in mm using a ruler. The results were recorded and interpreted according to the CLSI guidelines [13].

Statistical analysis

The data generated was analysed using IBM SPSS v25 software, a Pearson Chi-Square descriptive statistics method was used; the levels of

significant *p* value <.05 are considered as statistically significant.

Results

Both Gram positive and Gram negative bacteria were identified from urinary catheters and Gram negative bacteria had the higher prevalence than Gram positive. However, 47 isolates were identified of which 32 isolates were Gram negative bacteria (*E. coli* 15, *P. aeruginosa* 4, *P. mirabilis* 4, *P. vulgaris* 5, *K. pneumonia* 3) while 15 were Gram positive bacteria (*S. aureus* 11 and *S. saprophyticus* 4). The sensitivity pattern of the Gram negative bacteria isolated during the study is described in **table (1)**. The sensitivity of the identified bacteria to the tested antibiotics ranged from 20% to 100% while the resistant pattern ranged from 13.3% to 100%. The result showed that none of the isolates showed a complete resistant or sensitivity to all the antibiotics tested. Some Gram negative bacteria were multi drug resistant because they were resistant to more than one antibiotic tested. *Klebsiella pneumonia* was resistant to ampicillin, norfloxacin and tetracycline, *P. vulgaris* was resistant to ampicillin, gentamicin, cefotaxime and tetracycline. However, the pattern of sensitivity and resistant in *E. coli* varied, some were resistant while some were sensitive. *Pseudomonas aeruginosa* identified in this study were sensitive to ciprofloxacin and ceftriaxone. *Proteus mirabilis* was resistant to cefotaxime.

Furthermore, the Gram positive bacteria identified in the study were *S. aureus* and *S. saprophyticus*. The sensitivity pattern of the Gram positive bacteria identified in the study ranged from 9.09% in chloramphenicol for *S. aureus* to 100% in teicoplanin for *S. saprophyticus*. The resistant pattern ranged from 25% in gentamicin and vancomycin for *S. saprophyticus* to 100% in tetracycline for *S. saprophyticus* and 100% in gentamicin, penicillin and tetracycline for *S. aureus*. However, *S. aureus* were resistant to gentamicin, penicillin, and tetracycline but it showed some degree of variation in resistance and susceptibility against other antibiotics tested. *Staphylococcus saprophyticus* was found to be completely resistance to tetracycline and it also showed a complete susceptibility to teicoplanin as described in **table (2)**.

The sensitivity pattern of *E. coli* ranged from 33.3% in cefotaxime to 86.7% in nitrofurantoin while the resistant pattern ranged from 13.3% in nitrofurantoin to 73.3% in gentamicin.

The sensitivity and resistant pattern of *Escherichia coli* against the antibiotic tested is described in **figure (1)**. None of the antibiotics showed a complete sensitivity or resistance to all isolated *E. coli*, the degree of antibiotics resistances and susceptibility of *E. coli* against the tested antibiotics differ. However, nitrofurantoin had the highest susceptibility of 86.66% followed by tetracycline and ceftriaxone which had 73.33% each while gentamicin showed the highest resistant of 74% followed by cefotaxime with 66.67%.

Pseudomonas aeruginosa were sensitive to the tested antibiotics ranging from 25% in gentamicin and tetracycline to 100% in ciprofloxacin and ceftriaxone. The resistant pattern ranged from 25% in ampicillin and norfloxacin to 100% in nalidixic acid. **Figure 2** showed the result of antibiotics sensitivity on *P. aeruginosa* where ciprofloxacin and ceftriaxone showed a complete susceptibility to *P. aeruginosa* at 100% followed by ampicillin and norfloxacin with susceptibility of 75%. However, only nalidixic acid was found to be resistant to all the *P. aeruginosa* isolated in this study.

The pattern of sensitivity of *P. mirabilis* ranged from 40% in gentamicin and nalidixic acid to 100% in ceftriaxone; and the resistant pattern ranges from 20% in cephalothin, ciprofloxacin, and norfloxacin to 100% in cefotaxime. Various patterns of *P. mirabilis* sensitivity and resistant were observed in **figure (3)**. *Proteus mirabilis* showed a complete susceptibility at 100% to ceftriaxone but it was resistance at 100% to cefotaxime. However, the organism was susceptible to cephalothin and norfloxacin at 80%.

Proteus vulgaris was sensitive to the antibiotic tested ranging from 20% in tetracycline to 100% in ciprofloxacin. The resistant pattern also varied ranging from 20% in ceftriaxone and norfloxacin to 10% in ampicillin, gentamicin, and cefotaxime. **Figure 4** described the results of antibiotics susceptibility on *P. vulgaris* where the organism showed a complete susceptibility at 100% to cefotaxime and ciprofloxacin. This was followed by ceftriaxone and norfloxacin with 80%, while ampicillin and gentamicin showed a complete resistant 100% to *Proteus vulgaris*, followed by tetracycline with 80%.

The sensitivity pattern of *K. pneumonia* ranges from 33.3% in gentamicin and nitrofurantoin to 100% in cephalothin, ciprofloxacin, ceftriaxone, and nalidixic acid. The resistant pattern ranged from

33.3% in cefotaxime to 100% in ampicillin, norfloxacin, and tetracycline. Ampicillin, tetracycline and norfloxacin showed complete resistance to *K. pneumoniae* at 100% while cephalothin, ciprofloxacin and nalidixic acid showed complete susceptibility to *K. pneumoniae* as presented in **figure (5)**.

The antibiotic sensitivity pattern of *S. aureus* identified in the study showed the sensitivity range from 9.09% in chloramphenicol to 72.73% in ciprofloxacin. However, the resistant pattern range from 27.27% in ciprofloxacin to 100% in gentamicin, penicillin and tetracycline. Three of the antibiotics tested showed a complete susceptibility to *Staphylococcus aureus* as described in **figure (6)**. These antibiotics included gentamicin, penicillin and tetracycline. However, ciprofloxacin showed highest susceptibility against *S. aureus* with a

percentage of 72.72% followed by teicoplanin 54.54%.

Staphylococcus saprophyticus identified were sensitive to wide range of antibiotics ranging from 25% in ciprofloxacin, and penicillin to 100% in teicoplanin. While the resistant ranged from 25% in gentamicin and vancomycin to 100% in tetracycline. The result of antibiotic susceptibility revealed that only tetracycline showed susceptibility against the *S. saprophyticus* at 100%, while teicoplanin was found to be completely resistance to *S. saprophyticus*. Gentamicin, erythromycin and vancomycin showed resistance to *S. saprophyticus* with 75%. However, ciprofloxacin and penicillin were found to be susceptible against *S. saprophyticus* with 75% as described in **figure (7)** below.

Table 1. Result of Antibiotic susceptibility pattern of the isolated Gram negative bacteria.

Antibiotic	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>P. mirabilis</i>		<i>P. Vulgaris</i>		<i>K. pneumonia</i>	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Ampicillin	8(53.3)	7(46.7)	3(75)	1(25)	3(60)	2(40)	-	5(100)	-	3(100)
Cephalothin	6(40)	9(60)	2(50)	2(50)	4(80)	1(20)	3(60)	2(40)	3(100)	-
Gentamicin	4(26.7)	11(73.3)	1(25)	3(75)	2(40)	3(60)	-	5(100)	1(33.3)	2(66.7)
Ciprofloxacin	9(60)	6(40)	4(100)	-	4(80)	1(20)	5(100)	-	3(100)	-
Ceftriaxone	11(73.3)	4(26.7)	4(100)	-	5(100)	-	4(80)	1(20)	3(100)	-
Nitrofurantoin	13(86.7)	2(13.3)	2(50)	2(50)	3(60)	2(40)	3(60)	2(40)	1(33.3)	2(66.7)
Norfloxacin	9(60)	6(40)	3(75)	1(25)	4(80)	1(20)	4(80)	1(20)	-	3(100)
Nalidixic acid	8(53.3)	7(46.7)	-	4(100)	2(40)	3(60)	2(40)	3(60)	3(100)	-
Cefotaxime	5(33.3)	10(66.7)	2(50)	2(50)	-	5(100)	-	5(100)	2(66.7)	1(33.3)
Tetracycline	11(73.3)	4(26.7)	1(25)	3(75)	2(40)	3(60)	1(20)	4(80)	-	3(100)

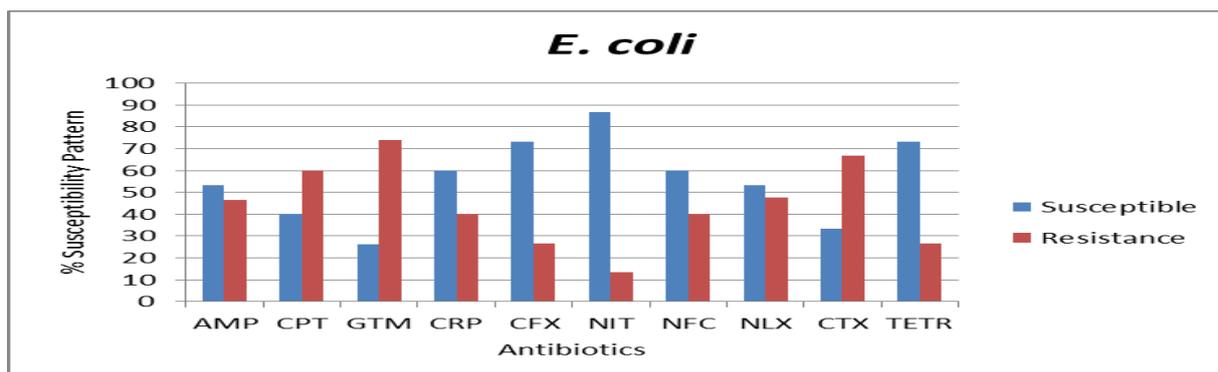
Pearson Chi-Square = 145.921^a, df = 63, $p = .000$. Key: R = Resistant, S = Susceptible

Table 2. Result of antibiotics susceptibility pattern of the isolated Gram's positive bacteria.

Antibiotic	<i>S. aureus</i>		<i>S. saprophyticus</i>	
	S (%)	R (%)	S (%)	R (%)
Gentamicin	-	11(100)	3(75)	1(25)
Ciprofloxacin	8(72.73)	3(27.27)	1(25)	3(75)
Penicillin	-	11(100)	1(25)	3(75)
Erythromycin	2(18.18)	9(81.82)	2(50)	2(50)
Chloramphenicol	1(9.09)	10(90.91)	2(50)	2(50)
Teicoplanin	6(54.55)	5(45.45)	4(100)	-
Tetracycline	-	11(100)	-	4(100)
Vancomycin	3(27.27)	8(72.73)	3(75)	1(25)

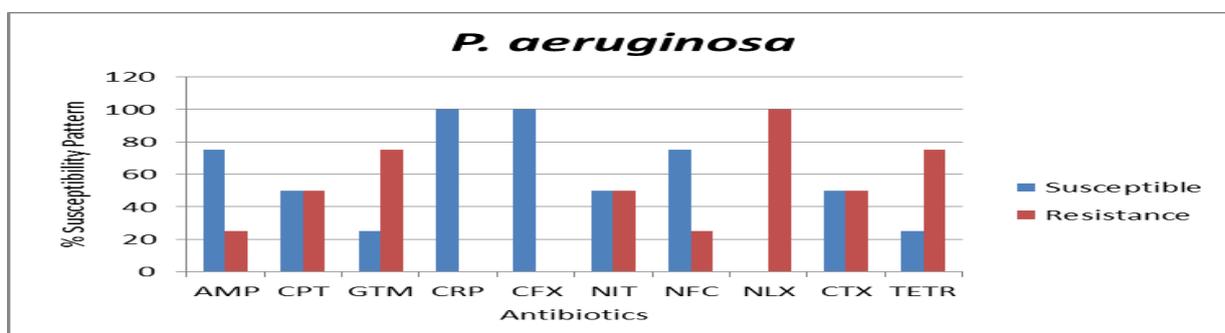
Pearson Chi-Square = 34.222^a, df = 24, $p = .081$. Key: S-Susceptible, R-Resistant

Figure 1. Antibiotics susceptibility against *E. coli*



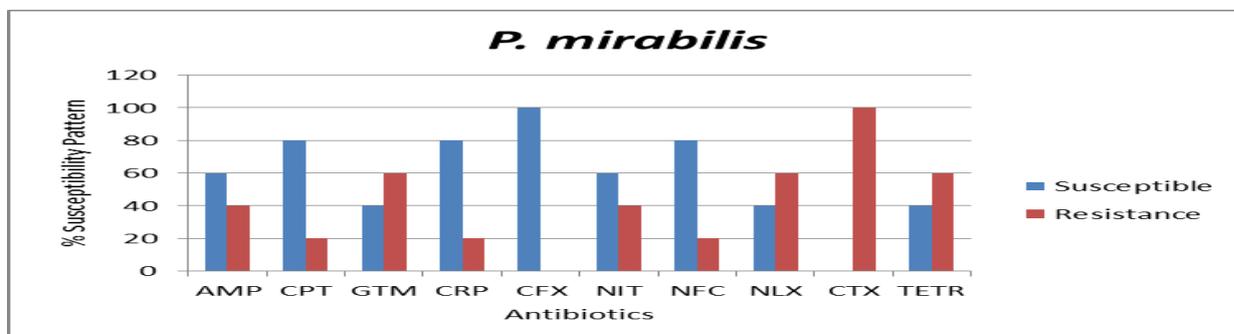
Key: AMP= Ampicillin, CPT= Cephalothin, GTM= Gentamicin, CRP= Ciprofloxacin, CFX= Ceftriaxone, NIT= Nitrofrontoin, NFC= Norfloxacin, NLX= Nalidixic acid, CTX= Cefotaxime and TET= Tetracycline.

Figure 2. Antibiotics susceptibility against *P. aeruginosa*.



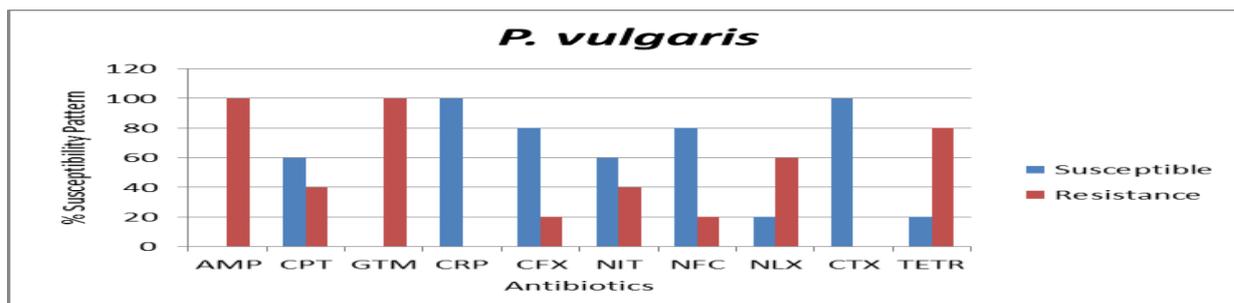
Key: AMP= Ampicillin, CPT= Cephalothin, GTM= Gentamicin, CRP= Ciprofloxacin, CFX= Ceftriaxone, NIT= Nitrofrontoin, NFC= Norfloxacin, NLX= Nalidixic acid, CTX= Cefotaxime and TET= Tetracycline

Figure 3. Antibiotics susceptibility against *P. mirabilis*.

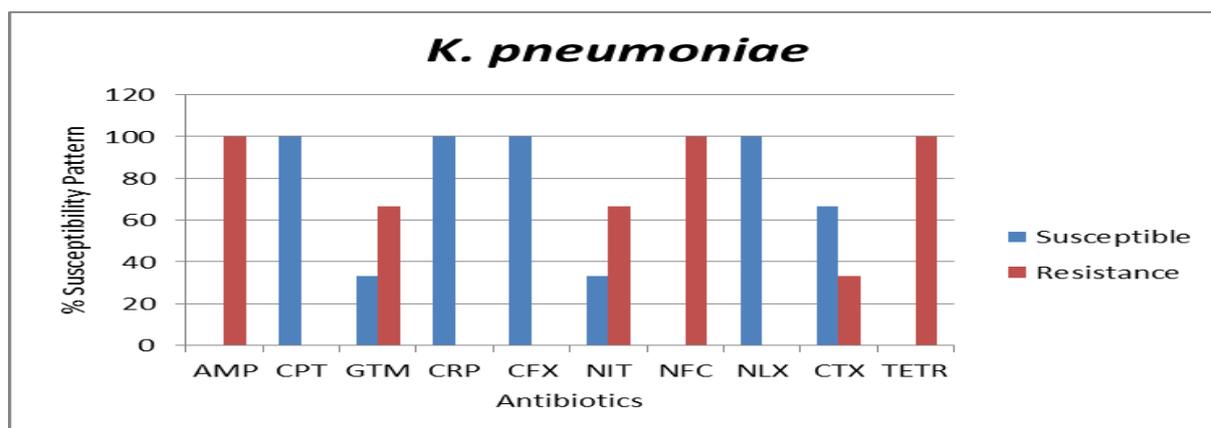


Key: AMP= Ampicillin, CPT= Cephalothin, GTM= Gentamicin, CRP= Ciprofloxacin, CFX= Ceftriaxone, NIT= Nitrofrontoin, NFC= Norfloxacin, NLX= Nalidixic acid, CTX= Cefotaxime and TET= Tetracycline.

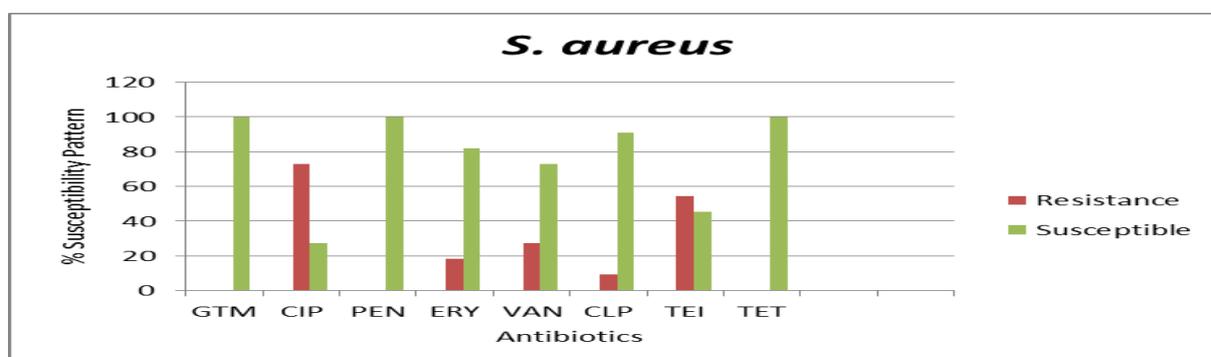
Figure 4. Antibiotics susceptibility against *P. vulgaris*.



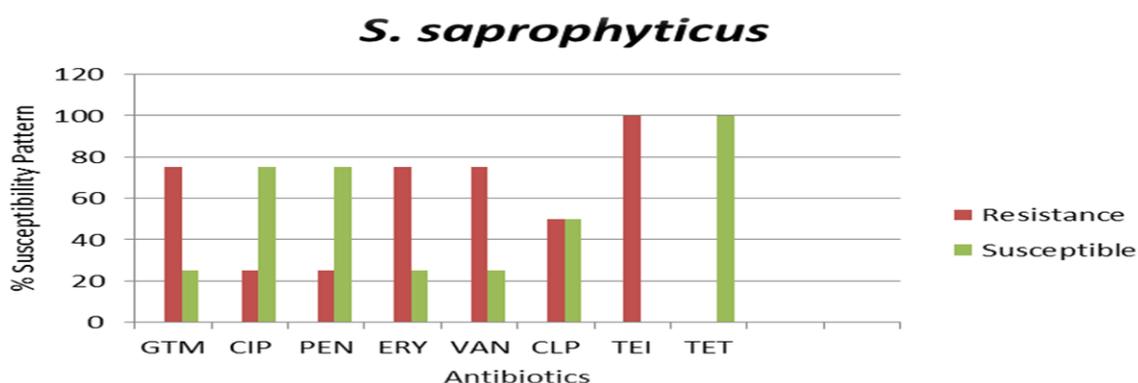
Key: AMP= Ampicillin, CPT= Cephalothin, GTM= Gentamicin, CRP= Ciprofloxacin, CFX= Ceftriaxone, NIT= Nitrofurantoin, NFC= Norfloxacin, NLX= Nalidixic acid, CTX= Cefotaxime and TET= Tetracycline

Figure 5. Antibiotics susceptibility against *K. pneumoniae*.

Key: AMP= Ampicillin, CPT= Cephalothin, GTM= Gentamicin, CRP= Ciprofloxacin, CFX= Ceftriaxone, NIT= Nitrofrontoin, NFC= Norfloxacin, NLX= Nalidixic acid, CTX= Cefotaxime and TET= Tetracycline

Figure 6. Antibiotics susceptibility against *S. aureus*.

Key: GTM= Gentamicin, CIP= Ciprofloxacin, PEN= Penicillin, ERY= Erythromycin, VAN= Vancomycin, CLP= Chloramphenicol, TEI= Teicoplanin, TET= Tetracycline.

Figure 7. Antibiotics susceptibility against *S. saprophyticus*.

Key: GTM= Gentamicin, CIP= Ciprofloxacin, PEN= Penicillin, ERY= Erythromycin, VAN= Vancomycin, CLP= Chloramphenicol, TEI= Teicoplanin, TET= Tetracycline.

Discussion

Antibiotic sensitivity pattern of bacteria identified in this study differs from one organism to another as well as Gram's positive or Gram's negative bacteria. Most of the bacteria identified are

resistant to more than one antibiotic; this can lead to infection with multidrug resistant pathogen. Both Gram's positive and Gram's negative causes UTIs and these organisms are also present in the catheters. The most common bacteria identified in the study

were *E. coli*. Other studies reported the most commonly encountered bacteria are Gram negative bacteria with *E. coli* being the most prevalent bacteria isolated in UTIs [15]. **Marami et al.** [16] in Ethiopia and **Veronica et al.** [17] in Italy observed *E. coli* as the most prevalent organism isolated from UTIs. The highest resistance rate of *E. coli* observed in our study was obtained against gentamicin with (74%), followed by cefotaxime (66.66%), nalidixic acid (47.67%), norfloxacin and ciprofloxacin (40%). This was in comparable with the result of a study obtained by **Perpetua et al.** [3] in Awka, Nigeria. Lower resistance was found in tetracycline and ceftriaxone by (26.67%) with nitrofurantoin (13.34%) having the least resistant antibiotics to *E. coli* isolates. Higher resistance was observed by **Akhilesh et al.** [18] on catheter associated UTIs (CAUTIs), where *E. coli* exhibited (98.75%) resistance to ampicillin followed by cefotaxime (96%), ciprofloxacin 82.5% and (47.5% & 22.5%) to gentamicin and amikacin respectively. However, frequent use of antibiotics may have resulted in these variations in antibiotic resistance.

All the *P. aeruginosa* were resistant to nalidixic acid, tetracycline, and gentamicin (75%). Ampicillin and norfloxacin (25%) was the least resistance antibiotic to *P. aeruginosa* isolates. Similar results were reported by **Perpetua et al.** [3] in Awka, Nigeria where they reported a resistance to gentamicin with 71.4%, nitrofurantoin with 85.7%. Moreover, they discovered 28.6% susceptibility to ciprofloxacin which is in contrast to our study where we observed a 100% susceptibility of *P. aeruginosa* to ciprofloxacin. In another study conducted by **Alsammani et al.** [19] they reported *P. aeruginosa* sensitive to ciprofloxacin with (75%) and norfloxacin (68.8%) and this in contrast to our study where we observed that *P. aeruginosa* was resistance to norfloxacin. Moreover, this difference in the sensitivity may result from the differences in the concentrations of the antibiotics used and/or conditions of the test.

The present study revealed that all *P. mirabilis* isolated in the study were susceptible and resistance to ceftriaxone and cefotaxime respectively. However, lower resistance of *P. mirabilis* to the antibiotics was obtained in cephalothin, ciprofloxacin and norfloxacin with 20.00%. Moderate resistance was obtained against gentamicin, nalidixic acid and tetracycline. *Proteus mirabilis* was also moderately susceptible to ampicillin, and nitrofurantoin. This result is in

conformity with the study of **Alsammani et al.** [19] where they observed that *P. mirabilis* ciprofloxacin and norfloxacin (90%).

Proteus vulgaris isolated in the study were found to be resistance to ampicillin and gentamicin, but they were also found to be susceptible to ciprofloxacin and cefotaxime used in the study. Furthermore, lower resistance and susceptibility was detected by ceftriaxone & norfloxacin and nalidixic acid & tetracycline respectively. *Proteus* species showed (100%) resistance to ampicillin, gentamicin and cefotaxime by **Alsammani et al.** [19] while ciprofloxacin and norfloxacin (20%) were the least resistance to *Proteus* species.

Higher degree of resistance and susceptibility was obtained against *K. pneumonia*. The organisms were found to be completely resistant to three of the tested antibiotics which included ampicillin, norfloxacin and tetracycline. This is similar to the result obtained by **Godfrey et al.** [1] where they reported that ampicillin, norfloxacin and tetracycline showed (100%) resistance to *K. pneumonia*. Also, in our study *K. pneumonia* was susceptible to four of the tested antibiotics such as cephalothin, ciprofloxacin, ceftriaxone and nalidixic acid. Hence, these antibiotics (cephalothin, ciprofloxacin, ceftriaxone and nalidixic acid) can be used in the treatment of CAUTI that involved *K. pneumonia* in the study area. Our result was almost with those reported by **Alsammani et al.** [19] where they reported that *K. pneumonia* was sensitive to ciprofloxacin norfloxacin and nalidixic acid (90%).

Staphylococcus aureus is among Gram positive bacteria isolated in this study and it is the second most common organism isolated from CAUTI in this study. The antibiotic sensitivity test against the organism revealed that the organism was completely susceptible to gentamicin, penicillin and tetracycline. It was also found to be susceptible to chloramphenicol with 90.90%. This is in accordance with the result of **Ahmed et al.** [20] with a resistance of *S. aureus* to ampicillin (100%), tetracycline (73.33%) and ciprofloxacin (86.67%), but susceptible for gentamicin (93.33%), teicoplanin (58.97%) and vancomycin (66.67%). **Adane et al.** [21] also reported a resistance of *S. aureus* to tetracycline (75.6%).

A hundred percent (100%) resistance was observed by *S. saprophyticus* against teicoplanin; however, the organism was found to be susceptible against tetracycline (100%). Less resistance was

obtained against ciprofloxacin and penicillin. This was in contrast to the study conducted by **Adane et al.** [21], where high level of resistance (88.9–94.4%) for clindamycin and erythromycin, respectively was observed. In our study, *S. saprophyticus* was resistant to vancomycin (75%) and this is not in-line with the study of **Adane et al.** [21] who observed that *S. saprophyticus* was 100% sensitive to vancomycin.

The differences in the susceptibility and resistance patterns of the bacteria isolated from catheters associated with urinary tract infections observed in our study and that of other studies could result from the differences in the hospital setup, patients frequent use of antibiotics as well as the infection control policies by different health institutions.

Conclusion

Different bacteria were isolated in the study which comprises both Gram positive and Gram negative bacteria, with *E. coli* and *S. aureus* as the most frequent Gram negative and Gram positive isolates respectively. Most of the Gram negative bacteria were sensitive to ciprofloxacin, ceftriaxone, nitrofurantoin and norfloxacin. However, among the Gram positive bacteria *S. aureus* was sensitive to tetracycline, penicillin and gentamicin while *S. saprophyticus* was sensitive to tetracycline but resistant to teicoplanin. Hence, for CAUTIs in the study area, one or more of the above antibiotics can be used to treat the infection depending on the type of bacteria identified.

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Conflict of interest

The authors wish to declare that there is no conflict of interest regarding the work as well as in the manuscript preparation.

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