Determination of Beta-lactamase Producing Bacteria and their Antibiogram for Urethral Catheterized Patients in Federal Medical Center (FMC), Umuahia, Abia State, Nigeria

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Abstract: The increasing rates of catheter associated urinary tract infection (CAUTI) and the resistance in empirical antibiotic therapy became a threat to mankind. This study was centered on isolating the common organisms responsible for this menace and their antibiotic resistance and susceptibility pattern. A total of 1000 urine specimens from 1000 patients on urethral catheter were used in the study. The specimens were cultured, biochemical tests were done including API and also antibiogram conducted. The organisms were cured of plasmid and re-subjected to antibiogram which revealed susceptibility to 98% of the isolated organisms. Delineation at 0.05level of significance did not show homogeneity among the different strains. There is high level of resistance to antibiotics by the organisms isolated and no single antibiotic used in the study was able to eliminate all the isolates identified. There was 100% resistance by all the organisms to Cotrimoxazole, Ceftazidime (93%), Cefuroxime (93%), Gentamycin (67%), Cefixime (99%), Nitrofurantoin (89%), Ciprofloxacin (77%), Cotrimoxazole (100%), Cloxacillin (100%), Erythromycin (94%), Streptomycin (94%), Tetracycline (98%), Chloramphenicol (83%), and Augemetin (96%). Analysis of Variance (ANOVA) showed insignificant difference between means for the various parameters examined at $P \leq 0.05$. Beta-lactamase test conducted for the isolates showed 96% positive reaction confirming that quite a good majority of the isolates possess the enzyme beta-lactamase. The isolates were treated with acridine orange to eliminate the effect of plasmid and isolates re-subjected to antibiogram. Result shows almost 100% sensitive after plasmid curing. Resistance to antibiotics by the isolates are plasmid mediated. Therefore, we conclude that CAUTI organisms' resistance to antibiotics are mostly engineered by plasmids.
[Iwu, J. O. Chikere, C.B. and Otokunefor, T.V. **Determination of Beta-lactamase Producing Bacteria and their Antibiogram for Urethral Catheterized Patients in Federal Medical Center (FMC), Umuahia, Abia State, Nigeria.** *Nat Sci* 2016;14(6):69-78]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 9. doi:10.7537/marsnsj14061609.

Key word: CAUTI, Catheter, Beta-lactamase, Bacteria, Antibiogram, Urethral Catheterized Patients

1. Introduction

Aetio-pathogenic process in community-acquired and hospital acquired UTIs are not the same. Published data on this regard remains limited (Wilson and Gaido, 2004). Urinary tract, may be attacked by different kinds of organisms but most common are the gram-negative bacilli (Braunwald *et al.,* 2001; Wilson and Gaido, 2004). The most common and primary cause of infections of the urinary tract including cystitis is *E. coli* (Gunther *et al.,* 2001; Hynicwiez and Hynicwiez, 2001). From uncomplicated UTIs, it has been shown that *E. coli* accounts for 77.0% of isolates, a report recorded by the International survey of antimicrobial sensitivity of pathogens. Further studies have shown evidence that there is decline in the percentage of UTI caused by *E.coli* while other families of enterobacteriaceae takes the lead (Hynicwiez and Hynicwiez, 2001). However, study by Braunwald *et al.,* 2001 revealed other gram-negative rods, such as *Proteus, Klebsiella*, sometimes*Enterobacter*, represent minute percentage of non-complicated infections.

The above bacteria, in addition to *Serratia* and *Pseudomonas* are becoming more significance in recurrent infections, especially situations like urologic manipulation, calculi, and obstruction. In hospital environment, gram- positive cocci such as *Enterococcus* is most common isolates (Wilson and Gaido, 2004). Staphylococcus saprophyticus-novobiocin-resistant (Hynicwiez and Hynicwiez, 2001), however, 10 to 15% of acute symptomatic UTIs in the young females represent coagulase-negative species. However it should be noted that Isolation of S. aureus from urine may imply bacteremic infection of the kidney (Braunwald *et al.,* 2001).

In general, studies have proved that the following organisms are associated with CAUTI, *E. coli, K. pneumoniae, C. freundii, Proteus* (Johnson *et al.,* 1999), *Pseudomonas, Serratia,* coagulase negative *Staphylococcus and Enterococcus faecium* (Braunwald *et al.,* 2001). Statistics revealed that the frequency of *E. co*li is reducing currently over the years (Oelschlaeger *et al.,* 2002) 35.6%, 32.5% and 26.6%, while *Enterococcus* became second most

frequent, with increasing rate of 11.8%, 15.3% and 22.0%.

Patients with chronic indwelling urinary catheter harbors variety of mixed organisms. Often times two to five organisms may be isolated at any giving time ,most often those of gram- negative enterobacteriaceae, according to Oelschlaeger *et al.* (2002) such as "*E. coli, Klebsiella pneumoniae, Citrobacter* species and urease producing organisms including *Proteus mirabilis, Morganella morgani* and *Providencia stuartii*, also further gram-negative organisms include *Pseudomonas aeruginosa*, *Acinetobacter* species or *Stenotrophomonas maltophilia."* In addition, some gram positive organisms have been implicated, as recorded by Rijavec and Zgur (2008) mainly "coagulase negative *Staphylococci Enterococcus* species and group *B streptococcus."* Fungal organisms especially yeast may be isolated for patients on antimicrobial treatment, others include *Candida albicans*, *Candida glabrate* and *Candida tropicalis*.

The dynamic nature of organisms in patients with chronic indwelling catheter remains constant hence the old infecting organisms disappear spontaneously with the introduction of new organism, (Alhambra and Alos, 2004). How long microorganism last or persist depends on the specie according to Warren *et al.* (1982) for example, "*P. stuartii* last or persist longer than other infecting organisms while *Enterococcus* faecalis persist for the shortest time." In the group of *Proteus stuartii* strains. Mobley *et al.* (1988) recorded that "those with MR/K adhesion persist longer than those without this class of adhesion." CAUTI organisms exhibit more antimicrobial resistance, than those without indwelling catheters (Master and Joshi 2003). Thus, this study was centered on isolating the common organisms responsible for this menace and their antibiotic resistance and susceptibility pattern.

2. Materials And Method

2.1. Area of Study: This study was carried at Federal Medical Centre, Umuahia, Abia State. Umuahia is a cosmopolitan city located in the South East region of Nigeria mostly populated by indigenes and people from other parts of the country. The Federal Medical Centre, Umuahia remains the most attended public health facility in the state. Amongst other infections, urinary tract infections account among the major causes of hospital attendance in the State. The hospital contains approximately 1,800 beds with many sub-specialties like ENT unit, O&G unit, Surgery, Medicine, Peadiatrics, mental health, A&E, G.O.P.D and many branches of laboratory units. The hospital remains the first tertiary institution in Nigeria to have and do in-vitro fertilization (IVF) if not in West Africa or Africa at large. Abia State is estimated to be approximately 2.84million in 2006.

2.2. Sample collection: Specimen urine was collected from either the catheter tubes or the uribag to avoid missing organisms associated with the catheter. The urine samples were transported within 30 minutes of collection to the laboratory for analysis, patients with already existing urinary tract infection were excluded.

2.3. Culturing, Isolation and Identification: The samples were cultured using cystein, lycin, electrolyte deficiency (CLED) agar plate under strict aseptic procedure as described by Cheesbrough (2000). Using the calibrated loop method with a loop diameter of 4mm, 10ul of uncentrifuged specimen was transferred onto the agar plate and streaked without flaming the loop for isolation and incubated at 35^0c-37^0c for 24hr. The single colony type cultures were identified using standard microbiological methods up to genus/species level wherever applicable, combined with analytical profile index. Beta-lactamase test was conducted and isolates treated with acridine orange to remove the effect of plasmid while antibiogram repeated after plasmid curing.

2.4. Antibiogram of bacterial isolates: Antibiotic sensitivity testing was done following the Kirby-Bauer disc diffusion method according to the clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested were broad-spectrum penicillin, third generation cephalosporin, fourth generation cephalosporin, quinolones, tetracycline, macrolids, aminoglycosides and sulphonamides (Hemidia, India, ABTEX and ATEK UK).

2.5. Beta-lactamase test: Fifty bacterial isolates were selected for beta lactamase studies on the basis of their high resistance pattern towards the antibiotics tested. Beta-lactemase test, oxoid (nitrocefin) glaxo research 37/312. Oxoid Ltd., basing stoke, Hampshire, England was used. All the color change reactions to red were noted as positive beta-lactamase production test.

2.6. Plasmid curing: The isolates were grown in25g/l of Luria Bertani agar (Amresco USA), 10g/l tryptone, 5g/l yeast extract,10g/l Nacl and15g/l bacteriological agar. The agar were dissolved in 1000ml of distilled water, homogenized on hot plate magnetic stirrer, and subsequently sterilized at 121^oC for 15minutes in an autoclave. The agar was cooled to 45^oc and plates were poured, inoculated and incubated at 37^oc for 24h. Using subminimum inhibitory concentration of acridine orange, 0.3g was weighed and dissolved in 300ml of distilled water in a standard flask. Then freshly prepared isolates (24h culture) was emulsified with sterile normal saline, 2ml of nutrient broth (double strength) was prepared

in test tubes, A, B &C autoclaved at 121°c. Test tubes containing 2ml of nutrient broth(double strength) plus 1ml of acridine orange diluted (10%v/v from stock solution above), plus 0.1ml of the inoculums were prepared from test A. and 1.0ml was also transferred to test tube C containing 2ml of double strength nutrient broth hence reducing the dilution with less numbers of organism and less volume of acridine orange from the test tube C and 1.0ml was transferred to sterile test tube and filled with corresponding volume of sterile distilled water to make 10ml. The principle behind this test is to use the acridine to knock out plasmid factor responsible for the resistance of the isolates. The plasmid cured isolates were further subjected to antibiotic sensitivity test by disc diffusion method on Muller Hinton agar.

3. Results

A total of 1000 patients catheter urine specimen were used in this study as shown in Table 1, 80% were males and 20% females. Only 5% of the patients had catheter insitu for 1 – 3 days, 20% had 4 – 7 days, 36% had 8 – 14 days, 34% had 15 – 21 days while 5% had theirs for 22 – 30 days. Frequency and duration of catheterization increases with age (Table 1).

Table 1: Age and sex distribution of the patients with catheter/duration

Age Range (Years)	Gender		Catheter Duration (Days)
	Males (%)	Females (%)	
10-20	50(5.0)	0(0.0)	1-3
21-40	120(12.0)	80(8.0)	4-7
41-60	300(30.0)	60(6.0)	8-14
61-80	300(30.0)	40(4.0)	15-21
>80	30(3.0)	20(2.0)	22-30
Total	**800(80.0)**	**200(20.0)**	

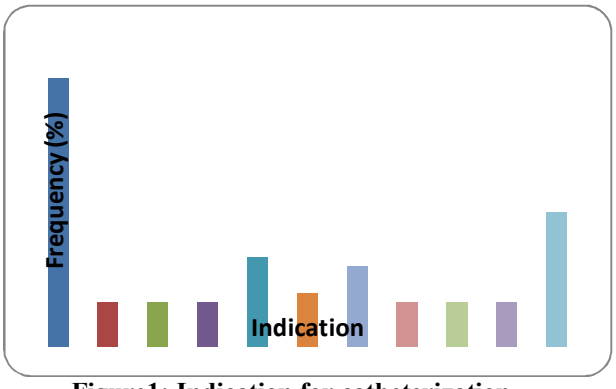

Figure1: Indication for catheterization

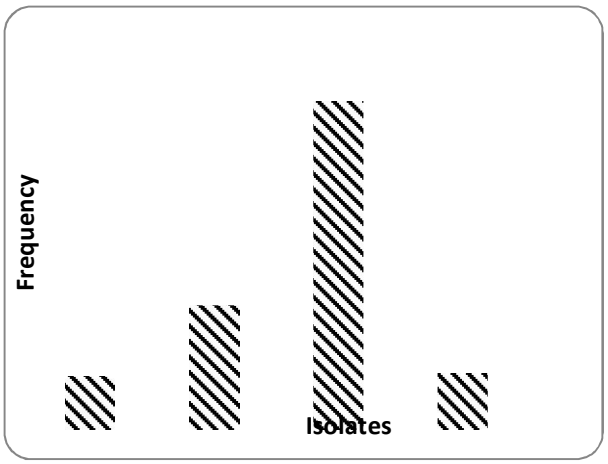

Figure2: Preliminary identification by gram staining

In Figure 1, it is clear that 30% of the cause of Catheterization was due to bladder outlet obstruction which invariably reflects to males because of the presence of prostrate at the bladder neck which can enlarge at any time to obstruct the flow of urine through the urethra. Other clinical conditions, top in Figure 1, are congestive cardiac failure 15%, acute abdomen 10% and cerebrovascular accident 9% are also age related. The remaining clinical conditions that lead to catheterization cut across to all ages and their occurrence are minimal as shown by their degree of percentage occurrence 5% and 6% (Figure 1).

Figure 2 reveals that a total of 58.4% of the isolates were gram positive while 10% were gram negative, tracing it down to Figure 5, shows that the gram positive were *Staphylococcus* spp. while the gram negative were *Pseudomonas spp., E. coli*, *Klebsiella* spp. and *Proteus* spp. In Figure 3, the distribution of bacteria by age reveals that there was increase rate of bacteria from age 41 – 80 and above, possibly a reflection of decline in immune status as one advanced in age (Figure 3). Bacteria distribution according to gender as shown in Figure 4, reveals high percentage of *Staphylococcus* spp. (68.5%) and *Pseudomonas* spp. (66.70%) more in males than in females with 31.5% and 33.3% respectively. Surprisingly, *Klebsiella pneumoniae* (24%), *Escherichia* coli (15%) and *Proteus* spp. (8.7%) are more in females than in males (20%, 0% and 14.3% respectively) as shown in Figure 4.

Total percentage of each organism involved in this study are shown in Figure 5, of which 85.4% reflects *Staphylococcus* spp., 7% for *Pseudomonas* spp., 4.3% for *Klebsiella* spp., 2.2% for *E. coli* and 7.2% for *Proteus* spp.

About 50 isolates subjected to beta-lactamase test, 48(96.0%) of the isolates were beta-lactamase

positive while only 2(4.0%) were beta-lactamase negative (Table 3). The API (analytical profile index) results in Table 4, shows slight variation for the species notably in isolates code numbers C1, C2, C3, Q6, O7, V10B, R5, O10B, S4, Q1, N2 and S7. This represents about 24% of the total isolates while 76% of the phenotypic identification agrees with the API.

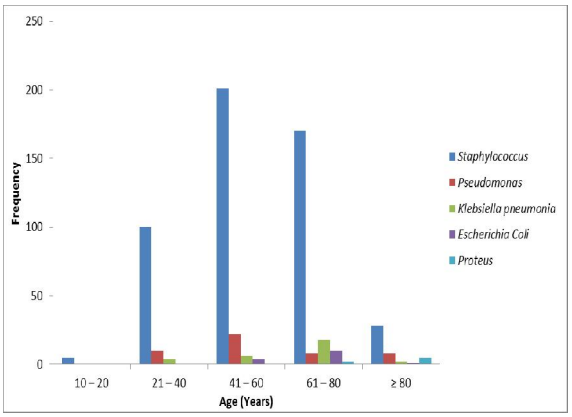

Figure3: Distribution of bacteria by age

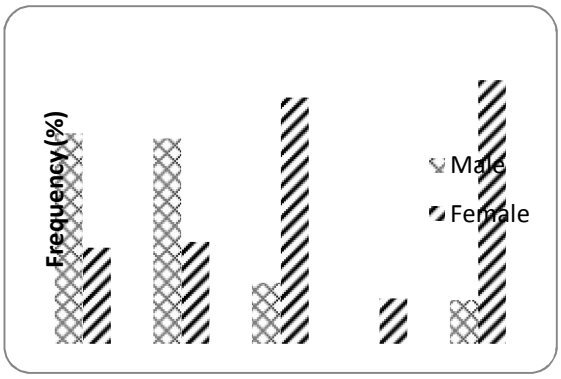

Figure 4: distribution of bacteria by gender

Table 5 shows susceptibility test of selected isolates before plasmid curing while Table 6 shows susceptibility test after plasmid curing. Table 5 reveals that apart from gentamycin, Ciprofloxacin, Augmentin, and Ofloxacin, E. *coli* was 100% resistance to other drugs used in this study while *Klebsialla* had similar behaviour apart from Ceftazidine, Gentamycin, Nitrofuretion, Chloramphenicol, Ofloxacin and Augmentin. Also *Proteus* had similar pattern apart from Gentamycin, Cefuroxime, Chloramphenicol and Ofloxacin. The whole organisms had 100% resistant to Cotrimoxazole, Cefuroxime, Streptomycin and Tetracycline (Table 5). Figure 7 highlighted the total percentage of resistance of the organisms to each drug: Cloxacillin 99.85%, Cefixime 98.98%, Augmetin 95.47%, Cotrimoxazole 100.0%, and Ceftazidine 92.98%. A look at the susceptibility test of the isolates in Table 5, before plasmid curing, confirms the high rate of resistance by these organisms to antibiotics. Resistance here is grossly alarming but surprisingly when the isolates were cured of plasmid (Table 6). Table 7 shows susceptibility test -ICOSA G-1-PLUS. Table 8 shows comparison of phenotypic and API results.

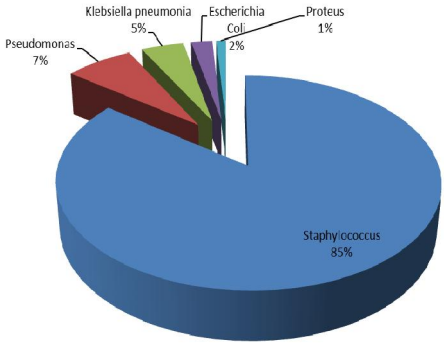

Figure 5. Percentage occurrence of the isolates

Table 2: Beta-lactamase Reaction

S/N	Code	Isolate	Reaction
1	C2	*Staphylococcus lentus*	+
2	D10	*Staphylococcus aureus*	+
3	C7	*Staphylococcus aureus*	+
4	T2	*Micrococcus varians*	+
5	D3	*Staphylococcus aureus*	+
6	D6	*Staphylococcus aureus*	+
7	S3A	*Enterobacter intermedius*	+
8	C3	*S. Warneri*	+
9	C4	*S. Xylosus*	+
10	N5	*E.coli*	+
11	G1	*E.coli*	+
12	O7	*Bacilis Coagulanis*	_
13	P4	*Micrococcus spp.*	+
14	Q5	*Proteus vulgaris*	+
15	M1	*Proteus mirabilis*	+
16	E5	*Proteus penneri*	+
17	Q4	*K. oxytoca*	+
18	V10B	*K. azaemune*	+
19	L9	*Klebsiella pneumoniae*	+
20	R5	*K. rhinoscleromatis*	+
21	O10B	*K. rhinoscleromatis*	+
22	B4	*K. rhinoscleromatis*	+
23	L7	*K. rhinoscleromatis*	+
24	T2	*Enterobacter aerogenes*	+
25	S4	*Enterobacter aerogenes*	+
26	SA	*Enterobacter aerogenes*	+
27	P3	*Enterobacter aerogenes*	+
28	M10	*Enterobacter intermedius*	+
29	2B	*Enterobacter intermedius*	+
30	S3A	*Enterobacter intermedius*	+

S/N	Code	Isolate	Reaction
31	T4	*Enterobacter cloacae*	+
32	D9	*K. oxytoca*	+
33	Q1	*K. ozaenae*	+
34	N2	*K. ozaenae*	+
35	S7	*Pseudomonas malleri*	+
36	Q3	*Pseudomonas stutzeri*	+
37	R6	*Pseudomonas cepaciae*	+
38	P4B	*Pseudomonas aeruginosa*	-
39	C5	*Pseudomonas aeruginosa*	+
40	K7	*Pseudomonas mendocina*	+
41	P5	*Pseudomonas mendocina*	+
42	F10	*Pseudomonas luteola*	+
43	V7	*Pseudomonas luteola*	+
44	V10A	*Pseudomonas luteola*	+
45	T1	*Pseudomonas oryzihabitans*	+
46	L1	*Proteus rettgeri*	+
47	C8	*Klebsiella pneumoniae*	+
48	Q6	*Bacillus subtilis*	+
49	L34	*Klebsiella pneumoniae*	+
50	Q7	*Klebsiella pneumoniae*	+

KEYS: + = POSITIVE; - = NEGATIVE

Table 3: API result for selected isolates

Specimen Code	API Identification
P4	*Micrococcus* spp
F5, C1, D6, D3, C7, Q6, B7, D10	*Staphylococcus aureus*
C4	*Staphylococcus xylosus*
C7	*Staphylococcus lentus*
C1	*Staphylococcus carnosus*
D2	*Kokuria varians*
Q6, M1	*Paenibacillus macerans*
O7	*Bacillus subtilis*
K3B	*Bacillus licheniformis*
N2, Q1, Q7, V103, L34, C8, R5, 010B, L9	*Klebsiella pneumonia*
D9, Q4	*Klebsiella oxytoca*
T4	*Enterobacter cloacea*
S3A, M10, 2B	*Enterobacter intermedius*
T2, SA, P3	*Enterobacter aerogenus*
S4	*Serratia fonticola*
K10B, D9, C5, P4B	*Pseudomonas aeruginosa*
P5, K7	*Pseudomonas mendocina*
Q3	*Pseudomonas stutzeri*
R6	*Burkhoderia cepacia*
S7	*Burkhoderia psedomallei*
V7, F10, V10A	*Chryseomonas luteola*
T1	*Flavimonas oryzihabitans*
N5, F5, G1	*Escherichia coli*
M1	*Proteus mirabilis*
Q5	*Proteus vulgaris*
E5	*Proteus penneri*
L1	*Providentia rettgeri*

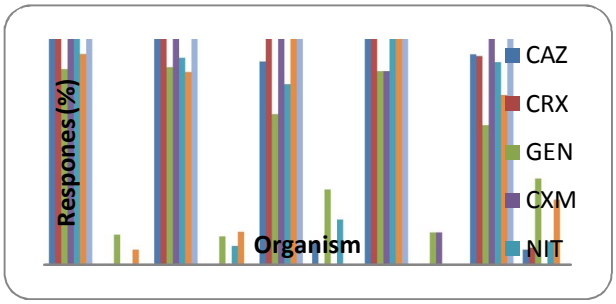

Figure 6a: Analysis of antibiotic susceptibility and resistant patterns of individual organism

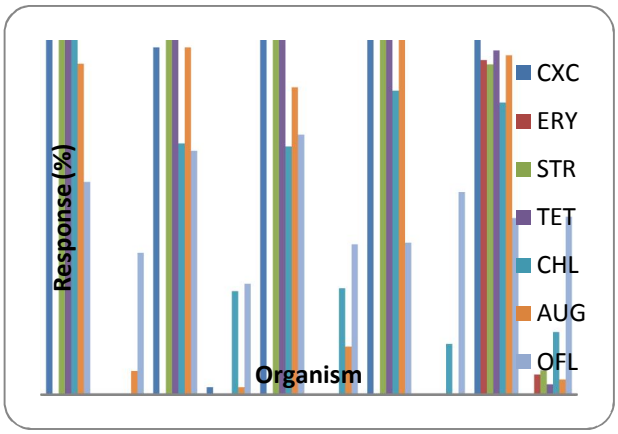

Figure 6b: Analysis of antibiotic susceptibility and resistant patterns of individual organism Continued

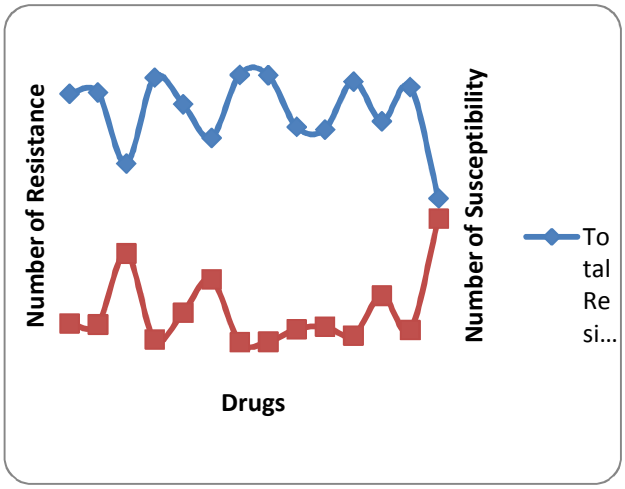

Figure 7: Total percentage resistance/ susceptibility of the organisms to each drug

Table 4: Percentage resistance/susceptibility of organism to each antibiotics

Code	*E.coli spp.*		*Pseudo. Spp.*		*Kleb. Spp*		*Proteus spp.*		*Staph. Spp*	
Drug	R	S	R	S	R	S	R	S	R	S
Ca2	15(100)	0(0)	48(100)	0(0)	27(90)	3(10)	7(100)	0(0)	545(93.32)	39(6.68)
CRx	15(100)	0(0)	48(100)	0(0)	30(100)	0(0)	7(100)	0(0)	540(92.47)	44(7.53)
GEN	13(86.67)	2(13.33)	42(87.50)	6(12.50)	20(66.67)	10(33.33)	6(85.71)	1(14.29)	361(61.82)	223(38.18
CXM	15(100)	0(0)	48(100)	0(0)	30(100)	0(0)	6(85.71)	1(14.29)	584(100)	0(0)
NIT	15(100)	0(0)	44(91.67)	4(8.33)	24(80)	6(20)	7(100)	0(0)	524(89.73)	60(10.27)
CPR	14(93.33)	1(6.67)	41(85.42)	7(14.58)	30(100)	0(0)	7(100)	0(0)	439(75.17)	145(28.83
COT	15(100)	0(0)	48(100)	0(0)	30(100)	0(0)	7(100)	0(0)	584(100)	0(0)
CXC	15(100)	0(0)	47(97.92)	1(2.08)	30(100)	0(0)	7(100)	0(0)	584(100)	0(0)
ERY	-	-	-	-	-	-	-	-	551(94.35)	33(5.65)
STR	15(100)	0(0)	48(100)	0(0)	30(100)	0(0)	7(100)	0(0)	544(93.15)	40(6.85)
TET	15(100)	0(0)	48(100)	0(0)	30(100)	0(0)	7(100)	0(0)	567(97.09)	17(2.91)
CHL	15(100)	0(0)	34(70.83)	14(29.17)	21(70)	9(30)	6(85.71)	1(14.29)	481(82.36)	103(17.64)
Aug	14(93.33)	1(6.67)	47(97.92)	1(2.08)	26(86.67)	4(13.53)	7(100)	0(0)	559(95.72)	25(4.28)
OFL	9(60)	6(40)	33(68.75)	15(31.25)	22(73.33)	8(42.37)	3(42.86)	4(57.14)	291(49.83)	293(50.17)

Table 5: Susceptibility test of selected isolates before plasmid curing using Hemidia disc (India)- ICOSA G-1-PLUS

Code	Organism	IPM	CP	TOB	MO	OF	SPR	LE	NX	COT	CL	NA	AMC	K	GAT	GEN	AK	S	CTR	CPD	TI
N2	*Klebsiella* spp.	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Q1		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Q7		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
C8		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R5		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
010B		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
L9		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
D9		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Q4		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
T4		R	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
S3A		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
M10		R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
2B		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
T2		S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SA		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R3		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S4		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
KLOB		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
D9	*Pseudomonas*	S	S	S	R	S	R	S	R	R	R	R	R	R	R	R	R	S	S	S	R
C5		S	S	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
P4B		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
P5		R	R	S	S	S	R	S	R	R	S	R	R	S	S	R	R	R	R	R	R
K7		S	R	R	S	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
Q3		R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R6		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S7		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
V7		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
F10		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
T1		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
N5		R	S	S	S	S	R	S	S	R	S	R	R	S	S	S	S	R	R	R	R
F5	*E.coli*	R	R	S	S	S	R	S	S	R	S	R	R	R	S	R	S	R	R	R	R
G1		R	S	R	S	S	S	S	S	R	S	R	R	R	S	R	R	R	R	R	R
M1		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Q5	*Proteus* spp.	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
L1		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

KEYS: IPM = Imipenem; CL = Colistin; CPD = Cetpodoxime; OX = Oxacillin; CP =Ciprofloxacin; NA = Nalidixic acid; TI = Ticarcillin; AZM = Azithromycin; TOB = Tobramycin; AMC = Augumentin; CEP = Cephalothin; AK = Amikacin; OF = Ofloxacin; K = Kenamycin; CD = Clindamycin; CLR =Clarithromycin; MO = Moxifloxacin; GAT = Gentifloxacin; E = Erythromycin; MET = Methicillin; SPR = Sparfloxacin; GEM = Gentamycin; P = Penicillin; AMC = Amoxiclave; LE = Lanfloxacin; AK = Amikacin; VA = Vancomycin; NV = Novobiocin;NR = Norfloxacin; S = Streptomycin; AMP = Ampicillin; TE = Tetracycline; COT = Cotrimoxazole; CTR = Ceftriaxone; C = Chloramphenicol; LZ = Linezolid

Table 6: Susceptibility test after plasmid curing with acridine orange

Code	Organism	IPM	CP	TOB	MO	OF	SPR	LE	NX	COT	CL	NA	AMC	K	GAT	GEN	AK	S	GR	CPD	TI
N2		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Q1		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Q7		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
C8		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
R5	*Klebsiella*spp	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
010B		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
L9		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
D9		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Q4		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
T4		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S3A		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
M10		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2B		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
T2		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
SA		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
R3		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S4		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
KLOB		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
D9	*Pseudomonas*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
C5		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Code	Organism	IPM	CP	TOB	MO	OF	SPR	LE	NX	COT	CL	NA	AMC	K	GAT	GEN	AK	S	GR	CPD	TI
P4B		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
P5		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
K7		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Q3		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
R6		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S7		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
V7		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
F10		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
T1		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
N5		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
F5	*E.coli*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
G1		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
M1		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Q5	*Proteus spp*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
L1	*Proteus* spp	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

KEYS: IPM = Imipenem; CL = Colistin; CPD = Cetpodoxime; OX = Oxacillin; CP =Ciprofloxacin; NA = Nalidixic acid; TI = Ticarcillin; AZM = Azithromycin; TOB = Tobramycin; AMC = Augumentin; CEP = Cephalothin; AK = Amikacin; OF = Ofloxacin; K = Kenamycin; CD = Clindamycin; CLR =Clarithromycin; MO = Moxifloxacin; GAT = Gentifloxacin; E = Erythromycin; MET = Methicillin; SPR = Sparfloxacin; GEM = Gentamycin; P = Penicillin; AMC = Amoxiclave; LE = Lanfloxacin; AK = Amikacin; VA = Vancomycin; NV = Novobiocin;NR = Norfloxacin; S = Streptomycin; AMP = Ampicillin; TE = Tetracycline; COT = Cotrimoxazole; CTR = Ceftriaxone; C = Chloramphenicol; LZ = Linezolid

Table 7: Susceptibility test -ICOSA G-1-PLUS

Code	CEP	CD	COT	E	GEN	P	VA	AMP	C	OB	AZM	AK	CLR	TEI	MET	AMC	NV	TE	OF	LZ
C1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
D6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
D3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
C7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Q6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
B7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
D10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
C4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
C1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
D2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
M1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
O7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
K3B	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 8: Comparison Of Phenotypic And Api Results

CODE	PHENOTYPIC ID	API ID
B7	*Staphylococcus aureus*	*Staphylococcus aureus*
D10	*Staphylococcus aureus*	*Staphylococcus aureus*
C7	*Staphylococcus aureus*	*Staphylococcus leubus*
D2	*Micrococcus various*	*Kocuria varius*
D3	*Staphylococcus aureus*	*Staphylococcus aureus*
D6	*Staphylococcus aureus*	*Staphylococcus aureus*
C7	*Staphylococcus canosus*	*Staphylococcus aureus*
C2	*Staphylococcus leutus*	*Staphylococcus aureus*
C3	*Staphylococcus warneri*	*Staphylococcus aureus*
C4	*Staphylococcus xylosus*	*Staphylococcus xynosus*
N5	*E. coli*	*E. coli*
G1	*E. coli*	*E. coli*
K3B	*Bacillus licheniformis*	*Bacillus licheniformis*
Q6	*Bacillus subtilis*	*Paenibacillus maceraus*
O7	*Bacillus coagulaus*	*Bacillus subtils*
P4	*Micrococcus sp.*	*Micrococcus sp.*
F5	*Staphylococcus leutus*	*Staphylococcus aureus*
Q5	*Proteus vulgaris*	*Proteus vulgaris*
M1	*Proteus mirabilis*	*Proteus mirabilis*
E5	*Proteus penneri*	*Proteus penneri*
L1	*Proteus rettgeri*	*Providential rettgeri*
Q4	*Klebsiella oxytoca*	*Klebsiella oxytoca*
V10B	*Klebsiella azaenae*	*Klebsiella pneumoniae*
L9	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*
C8	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*

CODE	PHENOTYPIC ID	API ID
R5	*Klebsiella rhinoscleromatis*	*Klebsiella pneumoniae*
O10B	*Klebsiella rhinoscleromatis*	*Klebsiella pneumoniae*
L3A	*Klebsiella rhinoscleromatis*	*Klebsiella pneumoniae*
Q7	*Klebsiella rhinoscleromatis*	*Klebsiella pneumoniae*
T2	*Enterobacter aerogenes*	*Enterobacter aerogenes*
S4	*Enterobacter aerogenes*	*Serratia torticola*
S9	*Enterobacter aerogenes*	*Enterobacter aerogenes*
P3	*Enterobacter aerogenes*	*Enterobacter aerogenes*
M10	*Enterobacter intermedius*	*Enterobacter intermedius*
2B	*Enterobacter intermedius*	*Enterobacter intermedius*
S3A	*Enterobacter intermedius*	*Enterobacter intermedius*
T4	*Enterobacter cloacae*	*Enterobacter cloacae*
D9	*Klebsiella oxytoca*	*Klebsiella oxytoca*
Q1	*Klebsiella ozaenae*	*Klebsiella pneumoniae*
N2	*Klebsiella ozaenae*	*Klebsiella pneumoniae*
S7	*Pseudomonas maltei*	*Klebsiella pneumoniae*
Q3	*Pseudomonas stutzeri*	*Pseudomonas stutzeri*
R6	*Pseudomonas copaciae*	*Pseudomonas cepaciae*
P4B	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
E5	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
K7	*Pseudomonas mendocine*	*Pseudomonas mendocine*
P5	*Pseudomonas mendocine*	*Pseudomonas mendocine*
F10	*Pseudomonas luteola*	*Chryseomonas luteola*
V7	*Pseudomonas luteola*	*Chryseomonas luteola*
V10A	*Pseudomonas luteola*	*Chryseomonas luteola*
T1	*Pseudomonas oryzihabitaus*	*Flavimonas oryzihabitaus*

4. Discussion

A total of 1000 patients catheter urine specimen were used in this study. Eighty percent were males and 20% females. It is clear that 30% of the cause of Catheterization was due to bladder outlet obstruction which invariably reflects to males because of the presence of prostrate at the bladder neck which can enlarge at any time to obstruct the flow of urine through the urethra. Bacteria distribution according to gender reveals high percentage of *Staphylococcus* aureus (68.5%) and *Pseudomonas aeruginosa* (66.70%) more in males than in females (31.5%) and 33.3% respectively. This may be as a result of majority of the sample size was males (80.0%) and females 20% or it reflects high level of contamination during the procedure of catheterization by the clinicians. Surprisingly, *Klebsiella pneumoniae* (24%), *Escherichia* coli (15%) and *Proteus mirabilis* (8.7%) are more in females than in males (20%, 0% and 14.3% respectively). This could be due to close anatomical relation of the female genetalia to the anus.

The distribution of bacteria by age reveals that there was increase rate of bacteria from age 41 – 80 and above, possibly a reflection of decline in immune status as one advanced in age. About 50 isolates subjected to beta-lactamase test, 96% of the isolates were beta-lactamase positive while only 2% were beta lactamase negative. This could explain the high level of resistance recorded in this study as almost 96% of the organisms had beta lactamase enzymes.

Staphylococcus is a common contaminant and this may explain the high level of percentage and a reflection of poor aseptic procedure adopted by the clinicians during catheterization. The API (analytical profile index) shows slight variation for the species notably in isolates code numbers C1, C2, C3, Q6, O7, V10B, R5, O10B, S4, Q1, N2 and S7. This represents about 24% of the total isolates while 76% of the phenotypic identification agrees with the API.

The whole organisms in this study showed 100% resistant to Cotrimoxazole, Cefuroxime, Streptomycin and tetracycline. This may be due to common drug abuse and the drugs are available to every chemist shops. This demonstrates high degree of resistance to commonly used antibiotics. A look at the susceptibility test of the isolates confirms the high rate of resistance by these organisms to antibiotics. Resistance here is grossly alarming but surprisingly when the isolates were cured of plasmid. This means that plasmid was the major resistant factor these organisms had in common and the genes for their resistance were mediated through plasmid.

Common organisms isolated from the study were *Staphylococcus spp., Klebsiella pneumoniae, E. coli,*

Proteus mirabilis, Serretia spp., Enterobacter spp. *and Providentia rettegeri and Pseudomonas aeruginosa*. This is in agreement with the work done by Braunwald *et al*. (2001), Benge (1998), and Johnson *et al*. (1999).The predominant organism found in the study was gram positive *Cocci* (*Staphylococcus* spp.). Though, this is not in agreement with the work done by Hynicwiez and Hynicwiez (2001) as well as Wilson and Gaido (2004) who reported that the predominant organism was *Enterococcus spp.*

According to Braunwald et al.(2001), many catheter associated urinary tract infection (CAUTI) isolated organisms display greater anti-microbial resistance than organisms that cause community acquired urinary tract infections (UTIS). This is true with the findings of this study (Figure 6a and 6b). Taiwo and Aderomumu (2005) reported that above 68% of the isolated pathogens showed resistance from two to nine antimicrobials and this is similar to the occurrence in this study.

In this study, the risk of CAUTI increases with age and catheter duration. This is a true reflection of findings by Kavathar and Kovazomuis (2003) and Maki and Tambyah (2000) who reported that the enteric gram negative organisms found in the catheterized urinary tract are those that are commonly associated with multidrug resistance. According to Oelschlaeger et al. (2002), most frequent gram negative *enterobacteriaceae* were *E. coli, Klebsiella pneumoniae, Citrobacter* spp. *and urease* producing organisms such as *Proteus mirabilis, Morgenella aeruginosa, Acintobacter spp. or Stenotrophomonas maltophilia.* This study is in agreement with previous reports.

Since the urine specimens were collected from different wards, the antibiotic resistance pattern varies. This is also in agreement with what was reported by Vogel and Rochette (2004). Drug abuse could account for the high degree resistance by these organisms as seen in Cotrimoxazole (100%), tetracycline (98%), and Cloxacillin (98%) which are more commonly available and a single dose of antibiotic leads to a greater risk of resistant organisms to that antibiotics in the person for up to a year. Also, Johnson *et al.* (1999) reported that insufficient long course of antibiotics causes a more severe infection that is more difficult to treat.

Resistance to Vancomycin and Methicillin were noted in this study, hence, the first documented strain with complete resistance to Vancomycin appeared in the USA in 2002. A steady increase in resistance to cephalosporins has been reported by Bradford (2006) which is also a reflection on what was reported in this study (Figures 6a- 6b and Tables 5-7).According to Paterson and Bonomo (2005), beta-lactamases are inhibited by Clavulenic acid, Sulbactam and Tazobactam. But this was not so in this study as Augmentin was resisted by some of the beta-lactamase producing organisms. Possibly, these organisms may have gotten other factors apart from beta-lactamase enzyme to resist Augumentin.

5. Conclusion

This study has established the fact that no single antibiotic used in this study was potent enough to eliminate all the organisms isolated. Almost all the isolated organisms possess plasmid for their effective resistance to antibiotics. Drugs like Cotrimoxazole and tetracycline should no longer be used in treating CAUTI as they have almost 100% resistance by all the isolates. CAUTI organisms are highly resistance to the commonly used available antibiotics even to the more potent ones and therefore could be enlisted as super bug. CAUTI organisms resistance to antibiotic is beta-lactamase mediated. Treatment of CAUTI should be based on sensitivity results since CAUTI organisms are turning up to be superbug. Catheterization should be inserted when it is absolutely needed to prevent the risk of CAUTI. Aseptic procedure during catheterization should be a golden rule in all manner of uretheral catheterization; therefore the procedures should be left for professionals. Weekly change of Catheter for those on long time *in situ* should be adopted to prevent CAUTI since catheter duration is one of the risk factors for CAUTI. Prescription of antibiotics should be able to capture the correct dosage and duration to prevent development of resistance by CAUTI organisms.

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4/19/2016