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Antibiogram Audit of Catheter-associated Uropathogenic Isolates in University of Calabar Teaching Hospital, Calabar, Nigeria: Advocating Laboratory-based Prescription Practices

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Authors' contributions

This work was carried out in collaboration among all authors. All liabilities therein pertaining to the content shall be borne by us. The study was conceptualized by authors GIO, UEE, SNU and AAI. Manuscript was written and designed by author GIO, vetted by authors EMB, SNO, AAI, UEE and SNU. Data were collected by all and analyzed by author AAI. All authors read and approved the final manuscript.

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ABSTRACT

Background: The most important risk factor to developing UTI is the presence of an indwelling urethral catheter. Eighty percent of nosocomial UTI was reported to be caused by urethral catheterization. UTIs in health care institutions and in those with frequent antibiotic exposures were frequently caused by multi- drug resistant pathogens. This study sought to determined the antibiogram of isolates from catheterized patients with UTIs with a view to establishing if there were justifications for empiric treatment of this condition in the study area in the absence of quality antibiotic formulary.

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Materials and Methods: Interviewer administered questionnaires were used to collect socio-demographic data. Specimens were cultured on 5% sheep blood agar (SBA), MacConkey and sabouraud dextrose agar plates and incubated at 37°c for 24 hours in ambient air. Significant bacteriuria was determined on growths from SBA. Growths were identified using standard biochemical techniques.

Results: The study established 74.3% (52) prevalence of CAUTIs amongst catheterized patients in the study area with 29 (41.4%) female dominance. Imipenem (93.9%) recorded the highest percentage susceptibility, followed by Amikacin (91.8%) and Piperacillin/ tazobactam (88.8%). *E. coli* 17(32.7%) was the dominant isolate. Extended spectrum β-lctamase prevalence was 23(44.2%) and MRSA 2(3.8%). There was significant statistical relation between ESBL production and resistance to other classes of antibiotics.

Conclusion: There is high percentage prevalence of multidrug resistance (MDR) among isolates of CAUTIs in the study area. We therefore advocate laboratory based prescription practice and de-emphasized empiric treatment pending when there would be in a quality drug formulary founded on regular resistance surveillance.

Keywords: Antimicrobial audit; catheter-associated; prescription practice; empiric treatment.

1. INTRODUCTION

Urinary tract infection (UTI) is an infection involving any part of the urinary tract including urethra, prostate (in males), bladder, ureters and kidneys. It accounts for 40% of hospital acquired infections [1]. The most important risk factor to developing UTI has been the presence of an indwelling urethral catheter. Eighty percent of nosocomial UTI was reported to be caused by urethral catheterization while 5-10 % was attributed to genitourinary manipulations [2]. It has been reported that 12-16% of hospital in-patients would have urethral catheterization at some points during the course of hospital stay [3]. The daily risk of acquisition of UTI varied from 3-7% when an indwelling urethral catheter remained insitu [1,4]. There has been report of high infection rate coupled with isolation of polymicrobial nosocomial pathogens, associated with hospital acquired urinary tract infections [5] and this emphasizes the need for meticulous laboratory-based nosocomial infections surveillance as a guide to appropriate antibiotics prescription practice.

In nosocomial UTI, *Escherichia coli (E.coli)* has been the predominant isolate. There is however increase prevalence of *Pseudomonas , Proteus, Klebsiella, Staphylococcus* , *Streptococcus* and *Enterococcus species* in hospital acquired UTI [6,7]. There have been reports of emerging changes in the proportion and antibiotic sensitivity patterns of these uropathogens [8]. Amongst hospitalized children in Ibadan, South-West Nigeria *Klebsiella pneumoniae* was the predominant organism (52.8%), followed by *E. coli* (25%) [9].

UTIs in- patients in health care institutions and those with frequent antibiotic exposures are frequently caused by multi- drug resistant pathogens such as extended spectrum beta-lactamasee (ESBLs), carbapenemase producing *Enterobacteriaceae* and methicillin resistant *Staphylococci* (MRSA) [10]. ESBLs are enzymes that hydrolyse third generation cephalosporins, aztreonam but not cephamycins or carbapenems and which are inhibited by β-lactam inhibitors such as clavulanic acid [11]. Methicillin resistant *Staphylococcus aureus* (MRSA), also known as multi-drug resistant *Staphylococcus aureus* or oxacillin-resistant *Staphylococcus aureus* is a strain of *Staphylococcus aureus* that is resistant to β- lactam antibiotics including the anti penicillinase lactam antibiotics: methicillin, dicloxacillin, nafcillin and oxacillin. It is defined as a strain of *Staphylococcus aureus* with an Oxacillin MIC of ≥4mg/L(mcg/ml) [12]

Catheter associated urinary tract infection (CAUTI) could be asymptomatic [13] and so could rapidly progress to life-threatening septicaemia [14-16] and death. In-patient death rate in people with hospital acquired urinary tract infection is 2-3 times greater than in non bacteriuric patients [17]. Against this background and in view of the dominant contribution of prolonged urethral catheterization to hospital acquired UTIs, this study sought to determine the antibiogram of isolates from catheterized patients with UTIs so as to establish if there were justifications for empiric treatment of these conditions in University of Calabar Teaching Hospital, in the absence of a local laboratory based antibiotic formulary. The results of this study, in the long run were intended to stimulate

researches, targeted at generating data that would put in place a workable laboratory based local antimicrobial drug formulary for management of not just UTIs but bacterial infections in the hospital, generally. The specific objectives included determining the prevailing microbial agents of CAUTIs in University of Calabar Teaching Hospital, Calabar, determining the antibiotic susceptibility and resistance pattern of bacterial isolates of CAUTIs in the study area and determining the percentage prevalence of extended spectrum β-lactamase-producing Gram negative isolates and methicillin-resistant *Staphilococcus aureus* (MRSA) causing CAUTIs in the study area.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in University of Calabar Teaching Hospital (UCTH), Calabar, Cross River State between January 15th and March 6th, 2015. The hospital was a 260 bedded tertiary facility located in Calabar, the seat of Government of Cross River State. It had 28 departments out of which 10 were core clinical departments with in-patient facilities. The hospital was patronized by inhabitants of the state, adjoining states and countries.

2.2 Study Design

Catheter urine specimens were collected from in-patients in the wards who did not complain or showed symptoms of UTI 48 hours following presentation and who had been on uninterrupted urethral catheterization for a period ≥2 days with or without symptoms of urinary tract infection. Collected specimens were processed in the Medical Microbiology laboratory of the hospital.

2.3 Sample Size

Out of 229 patient admissions during the period of the study, 70 patients met the inclusion criteria and were so enlisted.

2.4 Samples Collection

Interviewer administered questionnaires were used to collect baseline data on patients' socio-demographic data including diagnosis, duration of catheterization, antibiotics treatment history, co- morbidities. About 10 ml of catheter urine was collected from each participant through the catheter port. The specimens were transported in ice packs to the laboratory for immediate processing.

2.5 Samples Processing

Specimens were cultured on 5% sheep blood agar (SBA), MacConkey and Sabouraud dextrose agar plates and incubated at 37^{0c} for 18 hours in ambient air. Significant bacteriuria was determined on SBA. Growths were identified using standard biochemical techniques [18].

Urinary tract infection was diagnosed in accordance with the Centre for Disease Prevention and Control (CDC) guideline [19] which defined indications for UTI as the presence of at least 2 of the following with no other recognized cause: fever, urgency of urination, dysuria, suprapubic tenderness with pyuria, positive urine culture for symptomatic or positive urine culture in the absence of symptoms for asymptomatic UTI. Any episode of UTI that was not present in the first 48 hours of admission but became apparent thereafter, following urethral catheterization was diagnosed as CAUTI.

2.6 Antibiogram

Antimicrobial susceptibility testing of isolates was carried out using the modified Kirby-Bauer disc diffusion method, based on the Clinical Laboratory Standard Institute (CLSI) guidelines [20]. *E. coli* ATCC25922 and *Staphylococcus aureus* ATCC25923 strains were used as controls for species of *Enterobacteriaceae* and *Staphylococcus* respectively.

Suspensions of isolates and control were made to 0.5% Mcfarland standard using sterile normal saline. Suspensions of isolates and control were inoculated on separate Mueller Hinton agar (MHA) plates to make thin lawns, appropriate antibiotic disks were implanted on the lawn and incubated for 18 hours in ambient air at 37°C. Antibiotics zones of inhibition were read and interpreted as sensitive, intermediate sensitive and resistant in line with CLSI guideline. *Enterobacteriaceal* isolates with cefotaxime, ceftaxidime and cefpodoxime zones of inhibition less than 27 mm, 22 mm and17 mm respectively were suspected to be ESBL producing as recommended by CLSI guidelines. The suspected isolates were screened and confirmed for ESBL production. All *Staphylococcus aureus* isolates, irrespective of in- vitro activity were screened for methicillin resistance using cefoxitin disk (30 µg) diffusion method.

2.7 Phenotypic ESBL-screening Using Double Disks Synergy Technique

Isolates suspected to be ESBL-producing based on reduced zones of inhibition to ceftazidime and cefotaxime, following susceptibility testing were screened using the Double disks synergy technique based on CLSI criteria. *E. coli ATCC35218* (positive) ESBL and *E. coli ATCC25922* (negative) ESBL controls were used to quality control the test.

Suspension of isolate suspected of ESBL-production and controls were made to the density of 0.5% Mcfarland standard with sterile normal saline and separately inoculated on Mueller hinton agar plates to form thin homogeneous lawns.

The 3 antibiotic disks were implanted on each lawn with Amoxicillin clavulanate disk in the centre and Ceftazidime and Cefotaxime disks placed on either sides of it at distances of 20mm center to center. The plates were then incubated for 18 hours at 35°C in ambient air.

Enhancement of zone of inhibition (clavaulanic effect) around Ceftazidime or Cefotaxime or both, on the side proximal to the Amoxicillin/clavulanate was indicative of positive screening test for ESBL-production.

2.8 Phenotypic ESBL - confirmation by Combined Disks Method

All isolates that passed as positive in the ESBL screening test were confirmed as such with the combined disks ESBL confirmatory test. E.coli ATCC35218 (positive) and E.coli ATCC25922 (negative) ESBL control trains were used to quality control the test.

Suspensions of isolate and controls were made to match the density of 0.5% Mcfarlan standard and used to inoculate the Mueller Hinton agar plate to form a thin lawn.

The paired and single antibiotic disks were implanted on the lawn, side by side about 30 mm apart and incubated for 18 hours in ambient air at 35°C.
At the end of incubation, the diameter of the zones of inhibition around each disk were measured. A difference in diameter of zone of inhibition between the single disk and its combined pair of ≥ 5mm was confirmatory of ESBL-production.

2.9 Phenotypic Screening for Methicillin Resistant *Staphylococcus aureus* (MRSA)

They were only 2 isolates of *S. aureus* in this study. The 2 were screened for Methicillin resistance irrespective of their *In vitro* antibiotic activities using *Staphylococcus aureus ATCC 33591 and Staphylococcus aureus* ATCC 25923 as positive and negative controls respectively. Three – five colonies of isolates and controls were separately emulsified in 3 ml of sterile normal saline to give suspensions that matched the density of 0.5% Mcfarland standard. Thin lawns of these suspensions were made separately on different Muller Hinton agar plates. A 30 µg Cefoxitin disk was placed in the centre of each of the lawns and incubated aerobically for 18 hours at 35°C.

Isolates with zones of inhibition ≤21 mm were interpreted as MRSA positive and those with zones of inhibitions ≥22 mm were interpreted as MRSA negative according to CLSI guideline.

2.10 Germ Tube Test

A colony of yeast isolate was emulsified in a microtube containing about 0.5 ml of sterile human serum. The suspension was incubated for 4hours after which a drop of the suspension was placed on a clean glass slide, covered with coverslip and examined for germ tubes formation (sprouting of pseudohyphae with no constrictions at the point of origin) which was indicative of *Candida albicans*.

2.11 Data Analysis

All data were analysed using SPSS statistical software version 20.0

3. RESULTS

3.1 Socio-demographic Data

Two hundred and twenty nine patients were admitted for the period of the study, out of which 70 patients met the inclusion criteria and were enlisted. Thirty five (50%) were males and 35(50%) were females, giving a male to female ratio of 1:1. Dominant age group of participants was (26-45) years of age followed by (46-65) years. As at the time of sample collection, 51(72.9%) participants were catheterized for 3-7 days (i.e <8 days) followed by 14(20%) who were catheterized for >13days. The shortest duration

of catheterization was 3 days while the longest was a duration of 17 days. Five(7.1%) of the participants were HIV positive (Table 1)

3.2 Microbial Agents of CAUTIs in the Study Area

This study established 74.3% (52) prevalence of CAUTIs amongst catheterized patients in University of Calabar Teaching Hospital, Calabar. The females 29 (41.4%) were more infected than men 23 (32.9%). There was no case of polymicrobial CAUTI. Forty four (84.6%) of the isolates were Gram negative, 4 (7.6%) were Gram positive bacteria while 4 (7.6%) were isolates of *C. albicans*. E.coli 17 (32.7.%) was the dominant isolate, followed by *Pseudomonas aeruginosa*13(25.0%) and *Klebsiella pneumoniae* 8(15.4%). There were also 2 (3.8%) isolates of *Staphylococcus aureus*, 4(7.6%) isolates of *C. albicans* and 1 isolate each of *Coagulase Negative Staphylococcus*(1.9%) and *Enterococcus faecalis* (1.9%) Eighteen samples (25.7%) samples yielded no growth (Fig. 1).

3.3 Antibiogram Profile

All the isolates, except *C. albicans* were tested for susceptibility against a battery of 11 antibiotics. Imipenem (93.9%) recorded the highest percentage susceptibility, followed by Amikacin(91.8%) and Piperacillin/ tazobactam (88.8%). Others were Cefepime (70.8%), Ceftazidime (46.9%), Ceftriaxone (28.6%), Gentamicin (38.8%) Amoxicillin clavulanate (32.7%) and Nitrofurantoin (30.8%) Fig. 2.

3.4 Multidrug Resistance Profile of CAUTI Isolates

This study reported a percentage ESBL prevalence of 44.2%(23) and 3.8%(2) prevalence of MRSA among the isolates from catheterized in-patients in the hospital. There was statistically significant association between ESBL-production and resistance to some non-cephalosporin classes of antibiotics among which were amoxicillin clavulante ($p< 0.05$), ciprofloxacin ($p< .05$), gentamicin ($p< .01$) and nitrofurantoin ($p< .01$)

Table 1. Socio-demographic data

Characteristics	No / % Frequency
Age group (years)	
11-25	14 (20)
26-45	28 (40)
46-65	21 (30)
>65	7 (10)
Total	70 (100)
Sex	
Male	35 (50)
Female	35 (50)
Total	70 (100)
Duration of catheterization (Days)	
<8	51 (72.9)
8-13	5 (7.1)
>13	14 (20)
Total	70 (100)
HIV Status	
Negative	65 (92.9)
Positive	5 (7.1)
Total	70 (100)
Previous Hospital admission	
No admission	11 (15.7)
Last 3 months	30 (42.9)
Last 6 months	9 (12.9)
Last 12 months	8 (11.4)
>12 months	12 (17.1)
Total	70 (100)

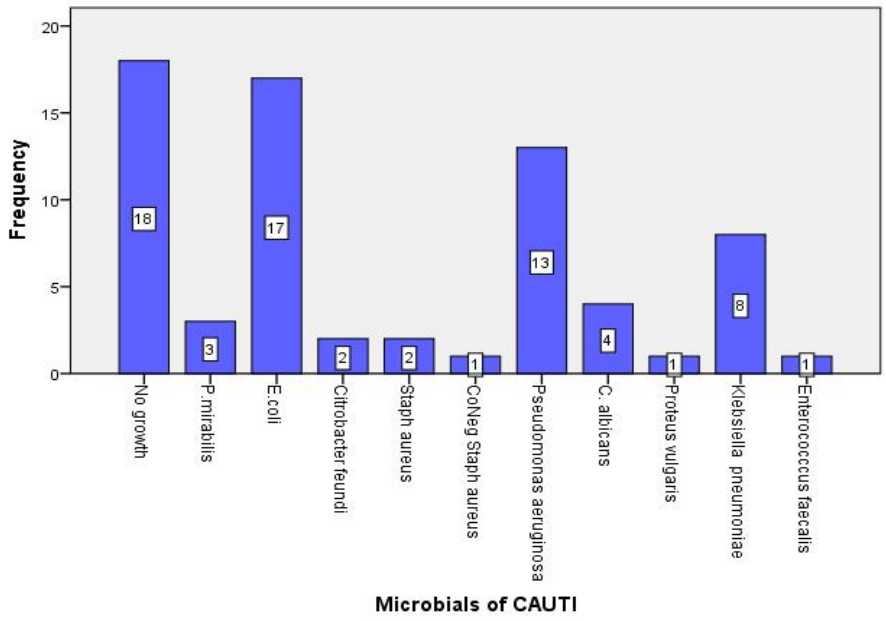

Fig. 1. Microbial agents of CAUTIs in the study area

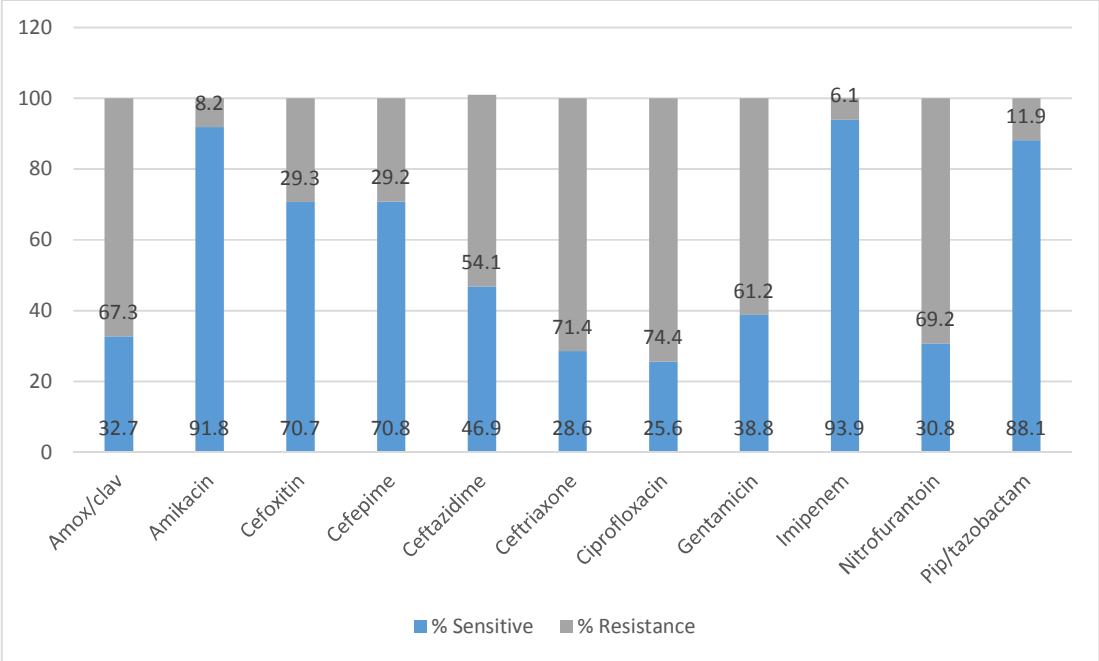

Fig. 2. Antibiogram profile of isolates of CAUTIs in the study area

4. DISCUSSION

Urinary tract infections represent at least 40% of hospital acquired infections with majority being catheter associated [21]. This study established 74.3% (52) prevalence of CAUTIs amongst participants in the study. The females 29 (41.4 %) were more vulnerable than men 23 (32.9%). This gender difference in prevalence might be due to the short urethra and the proximity of the

urethral orifice to the anal verge in females, making them more vulnerable to microbial contamination and hence UTIs. The finding is similar to a report of a study in Nepal [22] and another report by Nicolle's review [23] from 15 developing countries. There was no case of polymicrobial CAUTI, attributable to the fact that the durations of catheterization of participants in the study were in the short term range of <30 days [24] The isolates of CAUTI in this study were majorly *E. coli* (32.7%), *Pseudomonas aeruginosa*(25%), *Klebsiella pneumoniae* (15.4%), *C. albicans* (7.6%), *Proteus mirabilis* (5.8%), *Staphylococcus aureus* (3.8%), *Coagulase Negative Staphylococcus* (1.9%) and *Enterococcus faecalis (1.9%)*. This array of isolates conformed with the microbial growth pattern in short term catheterization [25] In all, *E. coli* (32.7%) was the dominant isolate in this study and this is similar to the result of a related study in Dhaka, Bangladesh [26] but different from the finding in a similar study [27] at the Lagos University Teaching Hopital, Lagos, Nigeria which reported *Klebsiella pneumoniae* as the dominant isolate.

Antimicrobial resistance is at the verge of driving humanity back to the dilemma of the pre-antimicrobial era,that probably was part of the reasons the Infectious Diseases Society of America (IDSA) remarked it as one of the greatest threats to human health globally [28]. In this study, the antibiotics with commendable sensitivity to uropathogens were imipenem (93.9%),amikacin(91.8%),piperacillin/tazobactam (88.1%), cefepime (70.8%), cefoxitin (70.7%). The under listed antibiotics showed remarkably reduced percentage sensitivity to agents of CAUTIs in the study. They included in paired values of percentage sensitivity to percentage resistance the following, ceftriaxone 28.6/71.4, ciproflocin 25.6/74.4, nitrofurantoin 30.8/69.2, amoxicillin/clavulanate 32.7/67.3, gentamicin 38.8/61.2, ceftazidime 45.9/54.1 (Fig. 2) This finding bears similarity to a study [22] at the Janamaitri Foundation Institute of Health Sciences, Nepal which reported these levels of resistance for similar antibiotics: imipenem 10.16%, amikacin 38.98%, piperacillin/ tazobactam 23.72%, nitrofurantoin 28.81%, gentamicin 42.37%, amoxicillin clavulanate 83.05%, ciprofloxacin 71.18% and ceftazidime 71.18%.

Out of the 52 isolates in this study, 25 (48.0%) were multidrug resistant, contributed by extended spectrum β-lactamases (ESBL) production 23(44.2%) and MRSA 2(3.8%). Although this level of ESBL prevalence is lower than 51.8% prevalence reported from a similar study from Italian Long term Care Facilities [29], it is a pointer to serious therapeutic challenges especially realizing that ESBL-elaboration recorded statistically significant association with resistance to other classes of antibiotics. Association between ESBL-production and resistance to other classes of antibiotics had also been reported by other studies [30-32]. The interplay between ESBL- production, its resistance associations and methicillin resistance among the CAUTIs isolates, portends significant therapeutic hiccups including treatment failures , long hospital stay, increased cost of treatment, loss of man-hours at work and death, just to mention a few. We therefore advocate laboratory based prescription practices and de-emphasized empiric treatment in the absence of a regularly reviewed antimicrobial formulary based on antibiotics resistance surveillance.

5. CONCLUSION

There was high percentage prevalence of multidrug resistance (MDR) among isolates of CAUTIs in the study area. Empiric treatment in this situation without a regularly reviewed drug formulary based on resistance surveillance was likened to shooting at a target in the dark, which carried obvious consequences. We therefore advocated laboratory based prescription practices and de-emphasized empiric treatment pending when there wiould be in place quality drug formulary, founded on regular resistance surveillance.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Inclusion Criteria: All in-patients on uninterrupted urethral catheterization for ≥2 days, who gave written informed consent and who did

not complain of or showed symptoms of UTI 48hours following admission.

Exclusion Criteria: (1) In-patients not catheterized. (2) Patients with established UTI on presentation. (3) those with urogenital fistulae, (4) Patients who met the inclusion criteria but refused to give consent to participate in the study.

ETHICAL APPROVAL

Ethical clearance was obtained from the Health Research Ethics Committee of the hospital. Aspects of the results of the study considered beneficial to patients were communicated to their respective managing medical teams.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

QUESTIONAIRE

Hospital number…… Managing surgical unit……Ward { }

1. Sex: Male{ }; Female { }
2. Date of Birth……
3. Occupation……
4. Reason (Indication) for admission…….
5. Indication for catheterization……
6. Previous hospital admission: None { }; last 3mnths { }; last 6mnths { }; last 12mnths { } ;> 12mnths { }
7. Antimicrobial Use History: None { }; last 3mnths { }; last 6mnths { }; last 12mnths { } ;> 12mnths { }
8.Antimicrobial was physician prescribed? yes { } no { }

9.UTI antimicrobial prophylaxis during catheterization? yes { } no { }
10. HIV status: Positive { }; Negative { }
11. Diabetic ?: Yes { }; No { }
12. prolonged steroids\ cytotoxic drug use ? Yes { }; No { }
13.Advance (2^{nd}-3^{rd} trimester) pregnancy? Yes { }; No { }
Date……..

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