Prevalence of *S. aureus* and *S. epidermidis* among patients with indwelling catheters and their antibiogram using some commonly used antibiotics.

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Abstract: The objectives of this study were to find the prevalence of *S. aureus* and *S. epidermidis* in urine samples of patients placed on catheter in Federal Medical Centre, Yola (FMCY) and State Specialist hospital, Yola (SSHY) and the efficacy of some commonly used antibiotics against the isolates of *S. aureus* and *S. epidermidis*. A total of one hundred and five samples (60 from SHHY and 45 from FMCY) were collected and inoculated into Cystine lactose electrolyte deficient (CLED) agar for isolation of Staphylococcal species. A total of seventy six presumptive Staphylococcal isolates were obtained on CLED agar and these isolates were identified using gram staining, morphological characteristics and standard biochemical tests. Serological studies revealed that out of 76 isolates, 56 were *S. epidermidis* (coagulase negative) and 20 were *S. aureus* (coagulase positive). Fifty one point eight percent (51.8%) of the isolates of *S. epidermidis* were sensitive to ceftazidime followed by ciprofloxacin (46.4 %) whereas 45% of the isolates of *S. aureus* were sensitive to ceftriaxone followed by cefotaxime and ciprofloxacin (40%). [De, N. and Godlove, M. Prevalence of *S. aureus* and *S. epidermidis* among patients with indwelling catheters and their antibiogram using some commonly used antibiotics. Journal of American Science 2010;6(11):515-520]. (ISSN: 1545-1003).

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Introduction

Urinary tract infections (UTIs) are the most common infections acquired in hospitals and long term care facilities. Several studies have estimated the incidence of health care associated UTIs at around 2-3 patients per 100 admissions (Kreiger *et al*., 1998). Catheter associated urinary tract infections (CAUTIs) perhaps the largest institutional reservoir of nosocomial antibiotic resistant pathogens. This could lead to complications such as pyelonephritis and bacteremia (Nwankwo *et al*., 2007). Glynn *et al*., 2007 have indicated that between 75 and 80 % of all health care associated UTIs follow the insertion of a urinary catheter, and a study investigating 40 English hospitals estimated that around 26% of all hospitalized patients have a urinary catheter inserted during their stay in hospital. Use of catheters is common in long term care facilities and many patients are catheterized for long period, thus increasing their risk of acquiring a CAUTI (catheter associated urinary tract infection). Ouslander *et al*., 1994 carried out a study on patients in nursing home and illustrated the problem of CAUTI in long term care of the elderly. During the one year study period, 80% of the patients had atleast one CAUTI and 48% of the patients had two or more CAUTIs. Nwankwo *et al*., 2007 carried out an investigation on catheter associated urinary tract infection in a tertiary health institution in Kano, Nigeria. The results show that out of 210 patients studied, 180 patients showed bacterial growth from the aspirated urine sample. The prevalence rates of the isolates for catheter tip and aspirated urine culture were *E. coli* (38.3%), 41.2%, *P. aeruginosa* 20%, 18.8%, *Proteus* sp. 12.7%, 11.8%, *S. aureus* 8.8%, 4.7%, *Streptococcus* sp. 1.1%, 7.1%, *C. freundii* 2.7%, 0%, *Candida* sp. 2.7%, 0% and *S. epidermidis* 1.1%, 0 % respectively.

Staphylococcus aureus causes a variety of suppurative infections and toxinoses in humans. It causes superficial skin lesions such as boils, styes and furunculosis, more serious infections such as pneumonia, mastitis, phlebitis, meningitis and urinary tract infections and deep seated infections such as osteomyelitis and endocarditis. *S. aureus* is a major cause of hospital acquired infection of surgical wounds and infections associated with indwelling

medical devices (Todar, 2005). *S. epidermidis* is known to cause infection of native heart valves, intravenous catheters, and artificial heart valves. It is a common skin resident that is sometimes responsible for endocartitis and infections of patients with lowered resistance (e.g. wound infections, surgical infections, urinary tract infections). It can cause peritonitis receiving peritoneal dialysis usually introduced via a break in the patient's skin and also causes infections in prosthetic joints ((Baron *et al*, 1994).

CoNS are the (coagulase negative *Staphylococcus*) quite essential pathogens of medical devices. The array of virulence factors produced by CoNS is meager compared with that of the virulence factors produced by *S. aureus*, but among these few factors are substances that promote bacterial adherence to and persist on foreign bodies. CoNS infection of intravenous catheter may or may not be accompanied by signs of inflammation at the site of catheter insertion, and the degree of systemic toxicity (including fever) ranges from minimal to moderately severe (Rupp and Archer, 1994).

Multidrug resistant Staphylococcus isolates have been recognized as one of the major challenges in control of hospital acquired infections and community associated infections. Bacteremia caused by *S. aureus* continues to be a common problem world wide and also the coagulase negative Staphylococcus and antibiotic sensitivity patterns are regarded with all seriousness in clinical practice and hospital acquired and community acquired infections. Martha *et al*. (2009) isolated MRSA from AIDS patients attending some public hospitals in Yola, Adamawa State, Nigeria. Shoba *et al*., 2005 conducted a survey work on prevalence of *Staphylococcus* sp. among hospital personnel, environment and their antibiogram with special emphasis on methicillin resistance. They reported that resistance to oxacillin was 13.84% among the 65 staphylococcal isolates. Yenda *et al*., 2010 isolated 60 isolates of *S. aureus* from patients attending State Specialist hospital and out of these 60 isolates, 85% of the isolates were resistant to ofloxacin and 80% of the isolates were resistant to ciprofloxacin.

FMCY and SSHY are the two largest hospitals in Adamawa State. Patients that need catheter devices or any prosthetic devices, generally attend these two hospitals. This present work was aimed at determination of prevalence of *S. aureus* and *S. epidermidis* isolated from patients with indwelling catheters and their antibiogram in these two hospitals.

Materials and Methods

Study area:

The study area selected for this study was Jimeta-Yola, Adamawa State. The selected hospitals were Federal Medical Centre, Yola (FMCY) and State Specialist hospital, Yola (SSHY).

Collection of samples:

According to Procter and Peters (1998), catheter urine samples should be collected not from the catheter bag but from the tube at upper connection. For collection of sample, the catheter tube was disconnected from the catheter bag and urine was collected into a wide mouth screw capped bottle and was covered after collection. Patient's age and gender were recorded for each of the sample. All the samples were analyzed within four hours of collection.

Isolation of Staphylococcus sp.:

A loopful of urine sample was inoculated on CLED agar and was incubated at 37^{o}C for 24 hours. The round cream and white colonies of 1-2 mm in diameter were collected and labeled. These were subcultured on blood agar and kept in refrigerator for identification purpose.

Identification of Staphylococcal isolates:

The appearance and color of the colonies on CLED agar and Blood agar and the diameter of the colonies were noted. Gram staining was done following the procedure as described in Benson, 2002. The isolates were also identified using different biochemical tests like catalase test, coagulase test, sugar utilization tests using sucrose, mannitol, trehalose and also novobiocin test using novobiocin at 5 µg/ml (Benson, 2002).

Isolates were then tested for coagulase production using Staphytect Plus test method. The reagents were purchased from Sanofi Diagnostic Pasteur, France and the method was followed as instructed by manufacturer. One drop of test latex reagent was dispensed onto one of the circles on the reaction card and one drop of control latex was dispensed onto another circle.

A loop was used to pick up 5 average-size of suspected staphylococcal colonies onto a culture media plate and mix this in the control latex reagent. The colonies were smeared in order to cover the circle. A separate loop was then used to proceed in the same way with the test latex.

The card was rocked for 20 seconds and agglutination was observed under normal lighting conditions.

A result was reported positive if agglutination of the blue latex particles occurred within 20 sec. A result was reported negative if no agglutination occurred and a smooth blue suspension remained after 20 sec in the test circle.

Antimicrobial susceptibility testing:

All 76 isolates were used for this test using streak plate method. Sensitivity disks containing conventional antibiotics like augmentin (5 µg/ml), ofloxacin (5 µg/ml), ceftriaxone ((30 µg/ml), ceftazidime ((5 µg/ml), cefotaxime (10 µg/ml), sparfloxacin (10 µg/ml) and ciprofloxacin (5 µg/ml) manufactured by BIOTECH LABS., England were used for sensitivity test. A loopful of growth of each isolate on blood agar was suspended in sterile water and then was diluted in steps of 1:10 to give turbidity equivalent to the 0.5 McFarland standard (a density of $1x10^8$ cells/ml) before inoculation (NCCLS, 2002). Diagnostic sensitivity test (DST) agar plates were inoculated with 0.5 ml of cell suspension of each isolate adjusted to $1x10^8$ using a sterile spreader. Sensitivity discs containing antibiotics were placed on the surface of each DST agar plate evenly seeded with the test organism and was incubated for 24 hrs. at 37^oC. The zone size of 20 mm or less for ciprofloxacin and 15 mm or less for ofloxacin, augmentin and sparfloxacin was considered as resistant and for other antibiotics (cephalosporin drugs) the zone size of 24 mm or less was considered as resistant for *S. epidermidis and S. aureus*.

Results

Description of samples:

A total number of one hundred and five samples were collected from FMCY and SSHY from patients placed on catheter. Out of 105 samples, 45 samples were from FMCY while 60 from SSHY. Out of the 45 samples from FMCY, 29 were from female patients while 16 from male patients. Out of 60 samples from SSHY, 38 were from female while 22 from male patients.

Isolation and identification of isolates:

Seventy six discrete colonies were isolated from CLED agar plates. The isolates produced cream, yellow or white colonies on CLED and blood agar plates. All the 76 isolates were gram positive cocci. The results of biochemical tests are listed in Table 1.

For isolates A1-A20, the agglutination occurred within 20 seconds signifying the fact that the isolates were *S. aureus*. For B1-B56, there was no agglutination verifying that B12-B56 were not isolates of *S. aureus*.

Results of antibiotic sensitivity test:

The results are shown in Table 2 and Table 3. The percent efficacy of different antibiotics is listed in Table 4.

Table 1: Biochemical characteristics of isolates

IN	S	Ca	Co	M	N	T	Organism
A1-A20	+	+	+	+	S	+	*S. aureus*
B1- B56	-	+	-	-	S	-	*S. epidermidis*

IN- isolate number, S- Sucrose, Ca- Catalase, Co- Coagulase, M- Mannitol, N- novobiocin at 5µg/ml.; T- Trehalose

Table 2: Zones of inhibition produced by antibiotics against isolates of *S. aureus*

IN	A	O	Ce	C	Cf	S	Ci	IN	A	O	Ce	C	Cf	S	Ci
A1	r	r	r	s	r	r	r	A11	s	s	r	r	r	r	s
A2	s	r	r	s	r	r	s	A12	r	s	s	r	s	r	s
A3	r	r	s	r	r	r	r	A13	s	r	r	r	r	s	r
A4	r	s	r	s	r	s	r	A14	r	s	s	r	r	s	r
A5	r	r	s	r	s	r	r	A15	r	s	r	s	r	s	s
A6	r	s	r	r	s	r	r	A16	r	r	r	s	r	r	s
A7	r	r	s	r	s	r	r	A17	s	r	r	s	r	s	r
A8	s	r	r	r	s	r	r	A18	r	r	s	r	s	r	s
A9	s	r	s	r	r	r	r	A19	r	r	s	r	r	s	r
A10	r	r	r	r	s	r	s	A20	s	r	s	s	s	r	s

IN-Isolate Number; A1-A20 isolates of *S. aureus*; A= augmentin; O= ofloxacin; Ce= ceftriaxone; C= ceftazidime; Cf= cefotaxime; S= sparfloxacin; Ci = ciprofloxacin; r = resistant; s = sensitive

Table 3: Zones of inhibition produced by antibiotics against isolates of *S. epidermidis*

IN	A	O	Ce	C	Cf	S	Ci	IN	A	O	Ce	C	Cf	S	Ci
B1	s	r	r	s	s	s	r	B29	r	s	r	s	s	r	r
B2	s	s	r	r	s	r	r	B30	s	r	s	s	s	r	r
B3	s	r	r	s	s	s	r	B31	s	s	r	s	s	r	r
B4	s	r	s	r	s	r	r	B32	r	r	s	s	r	r	s
B5	r	r	r	s	s	r	r	B33	r	r	s	s	r	r	s
B6	s	r	r	s	r	r	s	B34	r	r	s	r	s	s	r
B7	r	s	r	s	r	r	s	B35	s	s	s	r	r	r	s
B8	r	r	r	r	r	s	r	B36	s	s	s	r	r	r	s
B9	s	r	s	r	r	r	s	B37	r	r	r	s	r	r	s
B10	s	r	s	r	r	r	r	B38	r	r	s	r	r	r	s
B11	s	r	r	r	r	r	s	B39	s	r	r	s	s	r	r
B12	s	r	s	r	s	r	r	B40	r	r	r	r	s	r	r
B13	s	r	r	s	r	r	r	B41	r	r	s	s	r	r	s
B14	s	r	s	r	s	r	r	B42	r	r	s	r	s	r	r
B15	r	r	s	s	s	r	s	B43	r	r	r	s	r	r	s
B16	s	r	s	s	s	r	r	B44	r	s	r	s	s	r	r
B17	s	s	r	r	r	r	s	B45	r	r	r	r	r	r	r
B18	r	r	s	r	s	r	s	B46	s	s	r	r	r	s	r
B19	r	r	r	s	r	r	s	B47	s	r	r	s	r	r	r
B20	r	r	r	r	r	r	s	B48	s	r	r	s	r	r	s
B21	r	r	r	s	r	s	r	B49	r	r	r	s	r	s	r
B22	r	s	r	s	s	s	s	B50	r	r	r	r	s	r	r
B23	r	s	s	s	r	r	s	B51	r	r	r	r	r	r	s
B24	r	r	r	r	r	r	r	B52	r	r	r	s	s	s	s
B25	r	r	s	r	r	s	r	B53	r	s	s	s	r	r	s
B26	r	s	r	r	r	r	s	B54	r	r	s	r	r	r	s
B27	r	r	r	s	r	r	r	B55	r	s	s	r	r	r	s
B28	s	s	r	s	r	r	r	B56	r	s	s	r	s	r	r

IN-Isolate Number; B1-B56- isolates of *S.epidermidis*; A= augmentin; O= ofloxacin; Ce= ceftriaxone; C= ceftazidime; Cf= ceftriaxone; S= sparfloxacin; Ci = ciprofloxacin; r- resistant; s-sensitive

Table 4: Percentage efficacy of different antibiotics against isolates of *S. aureus* and *S. epidermidis*

Antibiotics	*S. aureus* No. of sensitive isolates	percentage efficacy	*S. epidermidis* No. of sensitive isolates	percentage efficacy
Augmentin	7	35.0	22	39.3
Ofloxacin	6	30.0	16	28.6
Ceftriaxone	9	45.0	23	41.0
Ceftazidime	7	35.0	29	51.8
Cefotaxime	8	40.0	22	39.2
Sparfloxacin	6	30.0	10	17.8
Ciprofloxacin	8	40.0	26	46.4

Discussion

The results from this study show that *S. aureus* and *S. epidermidis* were isolated from the urine samples of patients placed on catheter devices. Jernigan and Farr, 1993 reported that the catheter related infections are mainly caused by *C. albicans*, *S. aureus* and *S. epidermidis*. Some studies showed that *S. epidermidis* is a significant nosocomial pathogen, preferentially affecting immunocompromised patients and the cause of septicemia has been frequently associated with wounds and catheters or others indwelling devices (Huntton *et al*., 1985). CoNs are the most common pathogens complicating the use of intravenous catheters, hemodialysis shunt and grafts, cerebrospinal fluid shunts, peritoneal dialysis catheters, pacemakers wires and electrodes, prosthetic joints, vascular grafts and prosthetic valves (Rupp and Archer, 1994). The results obtained from this study also show that the prevalence of *S. epidermidis* (74%) is higher than that of *S. aureus* (26%) among patients placed on urinary catheter. This agrees with the findings of Buchman *et al*., 2007 who reported that coagulase negative staphylococcus infections are associated with medical devices and removal of such devices is often required for cure. Fidalgo *et al.*, 1990 showed that *S. epidermidis* is the casual agent of true bacteremia on the basis of microbiologic, epidemiologic and prognostic data on 60 episodes of *S. eptdermidis* bacteremia recorded in the hospital Covadonga of Oviedo, Spain during 1982-1986. Fifty one of SEB (*Staphylococcus epidermidis* bacteremia) were associated with indwelling devices, which in 44 cases were intravascular catheters; these included 23 central venous catheters and 21 peripheral intravascular catheters. The duration of catheterization ranged from 3 to 21 days for patients with central venous catheters and from 5 to 27 days for patients with peripheral intravascular catheters. For catheter insertion, rigorous attention should be given to aseptic techniques which involve washing of hands, application of 2% chlorohexidine to the site before catheter insertion, using of normal saline to flush the devices e.g. arterial catheters. Failure to follow these steps during catheter insertion might be responsible for the prevalence of *S. epidermidis* and *S. aureus* in urine samples of catheter patients in FMC, Yola and SSHY, Yola.

In regard to percentage efficacy of different antibiotics, two isolates of *S. epidermidis* (B24 and B45) were resistant to all the antibiotics tested and less than 50% of the isolates of *S. aureus* were susceptible to all the six antibiotics tested. A feature of *S. epidermidis* is the high rate of multiresistant strains. In this study we found that 60% of the isolates of *S. epidermidis* were resistant to augmentin; 81% were resistant to ofloxacin, 57% to ceftriaxone, 48% to ceftazidine, 54% to cefotaxime , 82% to sparfloxacin, 53% to ciprofloxacin. It has been shown that between 75 and 85% of all health care associated UTIs follow the insertion of a urinary catheter and a study investigating 40 English hospitals estimated that around 26% of all hospitalized patients have a urinary catheter inserted during their stay in hospital (Glynn et al., 2007). Ceftriaxone showed highest antibacterial activity against *S. aureus* isolates with percentage efficacy of 45% followed by ciprofloxacin, cefotaxime (40%) and ofloxacin and others (30%). This resistance might be attributed to the production of chemical substances and endotoxins by the isolates. Yenda *et al*., 2010 isolated 60 isolates of *S. aureus* from patients attending State Specialist

hospital and out of these 60 isolates, 85% of the isolates were resistant to ofloxacin and 80% of the isolates were resistant to ciprofloxacin. Fidalgo *et al.*, 1990 reported among all isolates of SEB, 50% exhibited resistance to five antibiotics namely Penicillin G, ampicillin, oxacillin, erythromycin and clindamycin. This study shows that less than 50% of isolates of *S. aureus* and *S. epidermidis* were susceptibile to quinolone antibiotics (Ofloxacin and sparfloxacin) and to some 3rd generation cephalosporins like cefotaxime and ceftriaxone.

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