

Full Length Research Paper

Susceptibility of nosocomial *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* to some antimicrobial drugs routinely used in Adamawa State Hospitals, Nigeria

A. M. El-Mahmood^{1*}, N. De¹ and A. B. Alo²

¹Department of Microbiology, School of Pure and Applied Sciences, Federal University of Technology, Yola, Nigeria.

²Department of Biological Sciences, School of Pure and Applied Sciences, Federal University of Technology, Yola, Nigeria.

Accepted 25 March, 2010

The presence of pathogenic *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* causing nosocomial infections to hospitalized patients in four large State hospitals in Adamawa State was investigated for a period of two and half years. One hundred and sixty isolates of each organism were collected from clinical specimens obtained from patients admitted into the hospitals for reasons other than the infection caused by these organisms. Though from each hospital 160 isolates of each organism were collected, there were some variations in the proportions of isolates collected per site at $p < 0.05$ level of significance. For *E. coli*, 27.5% isolates were obtained from surgical wound and urinary tract sites. *C. albicans* was the most common organism associated with urinary tract (35 - 42.5%) and catheter site infections (20 - 25%). Majority of *S. aureus* isolates obtained were from skin and soft tissue infections (25 - 35%). Antimicrobial susceptibility tests of the isolates performed by the disk diffusion method gave variable results. In case of *E. coli*, resistance to ampicillin (80%) and tetracycline (82.5%) were particularly high in Specialist Hospital, Yola (SHY), than the other hospitals. The susceptibility data showed that *S. aureus* isolated from Specialist Hospital Yola (SHY) was resistant to penicillin (65%), ampicillin (77.5%) and tetracycline (72.5%). In this study, a significant number of isolates of *C. albicans* isolated from Specialist Hospital Yola (SHY), were resistant to miconazole (32.5%), nystatin (30%) and itraconazole (27.5%). The study showed that there are some microorganisms causing nosocomial infections and are not susceptible to some antimicrobial drugs commonly used in the hospitals in Adamawa State, Nigeria. Proportion of the isolates susceptible to antimicrobial drugs depends on the size of the hospital and the type of medical procedures performed in the hospital.

Key words: Pathogenic, Adamawa State, nosocomial, infections, susceptibility, antimicrobial, resistant.

INTRODUCTION

Health care facilities, whether hospitals, nursing homes, rehabilitation centres, maternities and or outpatient departments are places where one seek refuge for medical problems, but they can also be places where one would have his/her health conditions worsened because of the likelihood of getting infected with more deadly

pathogens. Nosocomial infection is a global problem, affecting 5% of all hospitalized patients and in some clinical services such as intensive care units, up to 10% of the patients are infected (Madigan et al., 2000). World wide, over two million nosocomial infections are recorded each year, and that only a relatively small number of organisms cause majority of the infections at several sites (Lark, 2001). Most of the infections are polymicrobial with gram-negative bacilli predominating. The most frequent infections are those of surgical wounds, blood, urinary and gastro-intestinal tract

*Corresponding author. E-mail: elmahmud.abubakar33@gmail.com, elmahmuda@yahoo.com

infections. Factors facilitating the spread of nosocomial infections are impaired immunity, extremities of age, treatments with broad spectrum antibiotics, the ever increasing variety of medical and surgical routes of infection (for example, those patients who have open wounds or tube going into their body) and transmission of drug-resistant microorganisms among crowded hospital populations, where poor infection control practices may facilitate transmission (Amita et al., 2003). The spread of infection is greater as patients move from intensive care unit to general wards and then to the community or between hospital and nursing homes (Beaudin et al., 2004).

The impact of nosocomial infection on an individual or on a community are frequent hospital visits, greater length of hospital stay, high rate of illness, loss of productivity and death, straining of family and hospital budgets and extra time of hospital staff (Lark, 2001; Beaudin et al., 2004). Nosocomial infection divert financial resources that otherwise could be used for improving health and threatens the success of global efforts to combat the major infectious diseases of poverty and ignorance (Amita et al., 2003). Several reports have implicated *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* as causative agent of hospital acquired infections (Lark, 2001). These organisms do not have fastidious growth requirements and can grow at various temperatures and pH conditions prevalent in the hospital environment, and in addition, are able to exploit varieties of carbon and energy sources. These properties explain the ability of these pathogens to persist for a reasonable time in either dry or moist conditions in the hospital environment, thereby causing disease. These hardline posture combined with their intrinsic resistance to many antimicrobial agents, contribute to the organisms fitness and enable them to spread in the hospital environment (Beaudin et al., 2004). Records on agents of nosocomial infections and their susceptibility to antimicrobial drugs routinely used in the hospitals are scanty in Nigeria. This paper reports a study undertaken to investigate the prevalence and susceptibility of nosocomial *S. aureus*, *E. coli* and *C. albicans* in four large general hospitals in Adamawa State, Nigeria. It is hoped that the results obtained will serve a useful guide in the formulation of policies to contain the emergence and spread of infections in hospitals in the State.

This study did not include the traditional health care units where people may go and treat infections. It may be mentioned that a lot of patients run away from hospitals or are withdrawn by relatives in favour of traditional treatment, which is affordable and popular in Nigeria. The study has not included those smaller hospitals and clinics in Adamawa State where there are no or limited number of facilities and services (lack of surgical wards, maternity, intensive care units, laboratory services, pharmacy etc) as well as qualified health care professionals.

MATERIALS AND METHODS

Information regarding hospital settings were obtained from the hospitals, the Adamawa State Statistical Year Book (2003). Also some 500 questionnaires were prepared and distributed to hospital staff which were then collected back and analyzed. The information sought included the size of the hospital, staffing position, types of services rendered, diseased conditions that predisposed hospitalized populations to nosocomial infections, microorganisms frequently isolated, antimicrobial agents that were frequently used in the hospitals and their susceptibility patterns.

Selection of hospitals and sites

Based on a score point analysis, the 750 bed Specialist Hospital, Yola (SHY), the 600 bed General Hospitals Mubi (GHM), the 450 General Hospital Numan (GHN) and the 350 bed General Hospital Ganye (GHG) were selected for the study. Based on the responses obtained from the questionnaires, the selected wards were Surgical (male and female), Medical (male and female), Paediatric (medical and surgical), Intensive Care Unit, Nursery, ENT (Ear, Nose and Throat), Chest, Ophthalmic and Maternity. The sites selected for this study were surgical wound, catheter areas, blood, urinary and respiratory tract as well as skin and soft tissue sites.

Selection of microorganisms

Analysis of the information obtained from the questionnaires revealed that *S. aureus*, *E. coli* and *C. albicans* were the commonly isolated pathogens from specimens from hospitalized patients and were accordingly selected for inclusion in the study. A nosocomial isolate was defined as an isolate positive for any of the selected three organisms obtained from a site considered to be infected by the treating medical doctor (Lark, 2001). The organism must be isolated from a specimen taken for at least 48 h after hospitalization.

Selection of antimicrobials

Analysis of questionnaires gave the following drugs as the most frequently prescribed in the hospitals; ciproflox, norfloxacin, gentamycin, linocin, streptomycin, rifampicin, ofloxacin, erythromycin, chloramphenicol, ampiclox, tarivid, peflacin, augmentin, ciporex, nalidixic acid and co-trimoxazole. Others are miconazole, fluconazole, ketoconazole and nystatin.

Ethical consideration

An authorization to carry the study was obtained from the Ministry of Health and the Health Services Management Board, Yola as well as the Medical Officers in charge of the hospitals. The nature of the study was explained to each patient that was enrolled for the program and their consent obtained. Confidentiality was strictly maintained.

Selection of patients

Immediately after admission, specimens were taken from suspected patients for analysis in order to exclude those who were already harboring the organisms. Those that did not show the presence of any of the organisms were chosen as possible case patients for further observation of their clinical conditions. Specimens were collected from those patients after their appropriate hospital stay.

The parameters recorded for each patient included age, sex, race, tribe, provisional diagnosis, ward of hospitalization, name of the hospital, transfer from another hospital (clinic), or ward within the same hospital, Intensive Care Unit occupancy, underlying disorders, immunosuppressive therapy, severity of illness, invasive devices, dialysis, surgery, mechanical ventilation, catheters and antimicrobial drug therapy.

Collection of specimens

The period of collection of specimens from patients was between the months of January, 2003 to July, 2005. A request form was accompanied with each specimen collected from patient before or after hospitalization. Most specimens were collected before administration of any antimicrobial agents to the admitted patients. The times of collection of specimens were determined solely by the medical staff members and also the type of specimen to be collected from patient was determined by the nursing and medical staff members. For collection of stool samples, screwed-capped wide-mouthed specimen bottles were used. For urine, sterile universal bottles were used for collection of mid-stream urine samples. Most of the urine samples were collected from catheterized patients. Patients were advised to collect about 10 ml mid-stream urine samples in sterile wide-necked leak proof containers. Female patients were instructed to wash their hands before collecting mid-stream urine samples. For sputum collection, patients were instructed to provide deep coughed specimen and each of the specimens was expelled into a sterile container. For collection of throat and mouth specimens, the handle of a spoon was used to depress the tongue to examine the mouth for the presence of inflamed membrane, exudates or pus. The mucous membrane of the mouth was rubbed with a sterile cotton wool swab taking enough care not to contaminate with saliva. Each of the swab samples was transported to the Laboratory in container of Amies medium (Oxoid TS003A). Pus samples were collected at the time the abscesses were incised or ruptured naturally. Pus samples were also collected from surgical wounds just before dressing. About 5 ml of pus was collected from each of wound sites using a drainage tube into a sterile container and all the containers containing pus samples were taken to the Laboratory for isolation and identification purposes. Using a sterile cotton wool swab moistened in peptone water, the inner surface of the infected nose, ear or eye was swabbed gently and then the swabs were transported to the Laboratory in containers of Amies medium (Oxoid TS003A).

Specimens from skin and ulcerous sites were collected using swabs and the swabs were transported to the Laboratory in containers of Amies medium (Oxoid TS003A). Pus specimens from deeply ulcerated and necrotic tissues as well as fluid from blisters were collected using sterile needles and syringes before transporting to the Laboratory in sealed leak proof containers. For blood samples, 5 - 10 ml (for adults) and 1 - 2 ml (for infants and neonates) of venous blood was withdrawn from a patient using sterile syringe and needle and was immediately transported to the hospital laboratories for analysis. For collection of urogenital (vaginal swabs, cervical swabs, fluid and pus from genital ulcers) specimens, patients were requested not to urinate for at least 2 h before collection. Sterile swab moistened with saline was used to clean the urethral opening, after which the urethra was massaged from above downwards and the pus was collected on sterile cotton wool. For cervical specimens, the cervix was also washed using a sterile swab moistened with saline. A sterile cotton wool swab was pushed into the endocervical canal, gently rotated to obtain a specimen. For vaginal discharges and pus from genital ulcer specimens, the samples were collected using sterile cotton wool swabs. All the swabs were transported to the hospital Laboratories in containers of Amies Transport Medium (Oxoid TS003A). All the

specimens were properly labeled and were promptly delivered to the hospital laboratory for isolation and identification of the organisms selected for this study.

Isolation and identification of selected microorganisms

All the organisms were isolated and identified by the techniques routinely used in the study of clinical specimens (Potasmacher et al., 1979, Cheesborough, 2002). In hospital laboratories, stool samples were cultured on deoxycholate citrate agar (DCA) and corn meal agar (CMA). Urine samples were cultured on macConkey agar, blood agar, Sabouraud agar and Corn meal agar. Sputum samples and swab samples were cultured on blood agar, chocolate agar, sabouraud agar and corn meal agar. Blood agar (BA) plates, deoxycitrate agar (DCA) plates, macConkey agar plates were incubated at 37°C for 48 h and SDA plates and corn meal agar plates were incubated for 72 h at room temperature. Each of blood samples was inoculated onto heart infusion medium (Oxoid Ltd, England) contained in a special culture bottle and incubated for 72 h at 37°C. The discrete colonies were isolated and cultured on appropriate media and were kept at 6°C for identification purposes. The isolated colonies were subjected to gram staining reaction and those colonies that showed morphological and cultural characteristics of *S. aureus*, *E. coli* and *C. albicans* were selected for further identification purposes following the methods described by Nkang et al. (2008). The isolates were then further subjected to biochemical tests namely catalase and coagulase tests for *S. aureus*; indole and oxidase tests for *E. coli* and germ tube test for *C. albicans*. All the organisms were further confirmed using serological tests following standard procedure. Staphylase and Staphytec plus test for *S. aureus*; Microbat oxidase identification system and Dry spot Latex agglutination test for *E. coli* (Chessborough, 2002). *C. albicans* was confirmed using OBIS albicans test.

Preparation of cultures

The standardization of culture was done according to the method described by the Clinical and Laboratory Standard Institute (2006). Briefly 2 mm diameter colonies of the 18 h culture of an organism were picked with a sterile wire loop and immersed into a sterile bottle containing Mueller Hinton broth (Hi Media) and was incubated for 5 h. Normal saline was added gradually to it so as to compare the turbidity to that of 0.5 McFarland standard corresponding to approximately 1.0×10^8 cfu/ml. This was done for each of the test and control bacteria.

Antibiotic susceptibility testing

The antibacterial susceptibility testing of the isolates was done using the Kirby-Bauer disk diffusion method (Bauer et al., 1966) following the definition of the Clinical and Laboratory Standards Institute (2006) using antibiotics containing discs from Optum Ltd. Briefly, 20 ml of disc sensitivity test Mueller- Hinton agar (Difco Laboratories GmbH, Augsburg, Germany) was prepared and poured into sterile plates. The agar medium was allowed to solidify at room temperature on a flat bench. Then some few colonies of an 18 h culture of the isolates were streaked on the surfaces of the well-dried agar plates. Then some antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 h to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 35 - 37°C for 24 h. Zones of growth inhibition were then measured to the nearest millimetre and recorded. The mean of triplicate results was taken as the zone diameter. The antibiotics discs and the concentration used

were ciproflox (10 µg), norfloxacin (30 µg), gentamycin (10 µg), linocin (30 µg), streptomycin (30 µg), rifampicin (10 µg), ofloxacin (30 µg), erythromycin (30 µg), chloramphenicol (30 µg), ampicillin (30 µg), ampiclox (30 µg), tarivid (10 µg), peflacin (10 µg), augmentin (30 µg), ciporex (10 µg), nalidixic acid (30 µg) and cotrimoxazole (30 µg). Quality control on the susceptibility discs were performed using laboratory strains of *E. coli*, *Pseudomonas aeruginosa*, *S. aureus* and *Streptococcus faecalis* of known sensitivity. Resistant and intermediate isolates were grouped together for analysis in this study.

Preparation of antifungal discs

The antifungal susceptibility test for *C. albicans* was carried out using the method described by Achibald et al. (2004). Antifungal agents impregnated on paper discs were used for this purpose. A 6 mm cork borer was used to cut Whatman No. 1 filter paper in order to get a 6 mm diameter paper disc. The discs were placed in a bijoux bottle and sterilized by autoclaving. To make a concentration of 10 µg ml⁻¹ disc strength, 100 paper discs were placed in a bijoux bottle containing 1 ml of 1000 µg ml⁻¹ strength of miconazole and allowed to absorb the antifungal solution for 30 min. The dried antifungal discs were then kept in a closed bottle until required.

Antifungal susceptibility testing

The antifungal susceptibility test for *C. albicans* was carried out using the method described by Achibald et al. (2004) and Pfaller et al. (2006). Briefly, 20 ml of potato dextrose agar was prepared and poured into sterile plates. The agar medium was allowed to solidify at room temperature on a flat bench. The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of 0.5 McFarland Standard. The antifungal discs were then gently and firmly placed on the surfaces agar plates, which were then left at room temperature for 1 h to allow diffusion of the antifungal drugs into the agar medium. The plates were then incubated at 35 - 37°C for 48 h. Zones of growth inhibition were then measured to the nearest millimetre and recorded. The mean of triplicate results was taken as the zone diameter. The interpretive criteria for fluconazole and other drugs used in the disk diffusion tests were those described by Pfaller et al. (2005). The antifungal discs and the concentration used were fluconazole (10 µg/ml), ketoconazole (10 µg/ml) and nystatin (10 µg/ml). The same procedure was employed for fluconazole, ketoconazole and nystatin. On the basis of diameter of zone of inhibition, the isolates were grouped into well separated populations of susceptible and resistant pathogens (Lesch et al., 2001; Pfaller et al., 2001; Gupta et al., 2004). Quality control was performed with each test run in accordance with CSLI document M44-A (CLSI, 2006)

Statistical analysis

All data were analyzed with SPSS for Windows, version 16.0 (SPSS Inc. Chicago, Ill, USA). The trend χ^2 test for statistical comparisons between the groups and a $p < 0.05$ were considered as statistically significant.

RESULTS

Information generated from questionnaires, the Adamawa State Statistical year Book (2003) and the medical records of patients were used in the selection of study

areas, hospitals, wards, sites, patients, microorganisms, and antimicrobial drugs used in the different hospitals.

The prevalence of the organisms according to clinical diagnosis of patients used for collection of specimens are shown in Table 1. The results showed that the main diseased conditions for acquisition of hospital based infections in Adamawa State of Nigeria were surgery, urinary tract and respiratory tract diseases, accidents and burns, insertion of catheter, diabetes, cancer, renal and hepatic diseases as well as preterm neonates with low birth weight. Majority of the specimens for isolation of *E. coli* (19.32%), *S. aureus* (18.41%) and *C. albicans* (15.40%) were from patients that have undergone surgery. The proportion of isolates obtained from catheterized patients were 14.77% *E. coli*, 14.14% *S. aureus* and 18.30% *C. albicans*. From accident and burn patients, the proportion of the isolates were in the order of 11.59% *E. coli*, 18.99% *S. aureus* and 11.03% *C. albicans*. Similarly the proportion of isolates obtained from intensive care units were 7.73% *E. coli*, 10.08% *S. aureus* and 15.86% *C. albicans*, while the proportion of isolates obtained from patients diagnosed with cancer were 8.40% *E. coli*, 9.88% *S. aureus* and 10.80% *C. albicans*. At $p < 0.05$ level of significance, the chi-square calculated value was 101.25, which is far higher than the tabulated value of 26.29, therefore, the null hypothesis is rejected, and alternate hypothesis accepted. The proportion of the isolates obtained from patients admitted for diabetes, hypertension as well as renal and hepatic diseases followed similar pattern as described above. There was no significant relationship between the infectious sites within hospitals and the occurrence of the isolates obtained from the different hospitals and between sites within the same hospital at level of significance $p < 0.05$.

All the organisms were isolated and identified following standard microbiological procedures. *S. aureus* isolates were identified through catalase and coagulase tests and also by latex agglutination tests involving Staphylase and Staphytec Plus identification tests. *E. coli* isolates were identified by their reactions to oxidase and indole tests and also by using Microbact Identifications Oxidase tests. The isolates of the *E. coli* organisms were confirmed to be of the serotypes 026, 091, 0103, 0111, 0120, and 0145 and Verocytotoxin producing strains by latex agglutination tests involving the Dry spot *E. coli* seroscreen DR300M. The isolates of *C. albicans* were identified by their Germ-Tube production and colonial morphology on cornmeal agar and also using the colorimetric test of the Oxoid Biochemical Identification System (OBIS albicans).

Results in Table 2 showed the distribution of the isolated organisms according to hospitals and sites. For *E. coli*, 27.5% isolates were obtained from surgical wound and urinary tract sites; 17.5% were from catheter sites, 15% from blood sites, 7.5% from skin and soft tissue sites and only 2% from respiratory tract sites in

Table 1. Prevalence of the isolates according to predisposing factors.

Predisposing factor	Percentage prevalence						
	<i>E. coli</i>		<i>S. aureus</i>		<i>C. albicans</i>		Total (%)
	N	(%)	N	(%)	N	(%)	
Diabetes	35(42.87)	7.95	42(35.24)	8.14	18(29.7)	4.14	95(6.8)
cancer	37(42.7)	8.40	51(50.07)	9.88	47(42.21)	10.8	135(9.7)
Hypertension/stroke	42(31.63)	9.55	36(37.09)	6.98	22(31.27)	5.06	100(7.2)
Renal/Hepatic disease	29(28.15)	6.39	32(33.01)	8.20	28(27.83)	4.44	89(6.4)
Preterm neonates	34(49.02)	7.73	52(57.49)	10.08	69(48.47)	15.86	155(11.1)
Catheter site	65(68.95)	14.77	73(80.86)	14.15	80(68.17)	18.39	218(15.7)
Accident/burns	51(62.31)	11.59	98(73.07)	18.99	48(61.60)	11.03	197(14.2)
UTI/RTI	62(49.02)	14.09	37(57.49)	7.17	56(48.47)	12.87	155(11.1)
Surgery	85(78.13)	19.32	95(91.62)	18.14	67(77.24)	15.40	247(17.8)
Total	440		516		435		1391

$\chi^2 = 101.25$; $df = 16$ and tabulated value = 26.296. Figures in brackets are expected values.

case of Specialist Hospital, Yola (SHY). At $p < 0.05$ level of significance, the chi-square calculated value was 23.26, which is far higher than the tabulated value of 18.31. In General Hospital Mubi, 35.5% of *E. coli* were obtained from urinary tract sites, 30.0% from surgical sites, 15% from catheter sites, 10% from skin and soft tissue, 7.5%, from blood and 5% from respiratory tract. At $p < 0.05$ level of significance, the chi-square calculated value was 42.52, which is far higher than the tabulated value of 18.31, therefore the null hypothesis is rejected, and alternate hypothesis accepted. The situations in the other three hospitals are similar to those obtained in the Specialist Hospital, Yola. The distribution of the *S. aureus* and *C. albicans* isolates followed similar pattern to that of *E. coli*, though with slight variations depending on site and organism.

Of the total 480 organisms isolated (each of the three selected organisms having 160 isolates), 140 (87.50%) of *E. coli*, 138 (86.25%) of *S. aureus* and 37 (23.13%) of *C. albicans* were resistant to more than three antimicrobial agents. Results in Table 3 showed the prevalence of antifungal resistance in case of *C. albicans* according to hospitals in the State. In Specialist Hospital, Yola, 30% of the *C. albicans* isolates were resistant to nystatin, 27.5% to itraconazole, 32.5% to miconazole, 25% to fluconazole and 22.5% to ketoconazole. The results obtained from the General Hospital Mubi, General Hospital Numan and General Hospital Ganye were qualitatively similar to the Specialist Hospital, Yola. In general, the isolates were more resistant to itraconazole, fluconazole and miconazole than to nystatin and ketoconazole. At $p < 0.05$ level of significance, the chi-square calculated value was 69.65, which is far higher than the tabulated value of 21.03, therefore the null hypothesis is rejected, and alternate hypothesis accepted. An isolate of *C. albicans* was considered multi-drug resistant if it was resistant to at least three different drugs. *S. aureus* and *E. coli* were considered multi-drug resistant when they display

resistance to at least five of the tested antibiotics (Juuli, 2004; Abu-Hajier and Sharif, 2008) For *S. aureus* as shown in Table 4 in the Specialist Hospital, Yola, 77.5% of the isolates were resistant to ampicillin, 37.5% each to cloxacillin and erythromycin, 32.5% each to cloxacillin and erythromycin, 42.5% to gentamycin, 40% to streptomycin, 32.5% to ampiclox, 35% to lincocin, 37.5% to ciproflox, 25% to refampicin, 22.5% to norfloxacin and 17.5% to floxacillin. In general Hospital Mubi, the resistant rates for each of the drug was 67.5% for tetracycline, 60% for ampicillin, 52.5% for penicillin, 37.5% for chloramphenicol, 30% each for ampiclox, cloxacillin and ciproflox, 32.5 % each for streptomycin and lincocin, 27.5 % each for rifampicin and gentamycin, 25% for floxacillin and 17.5% for norfloxacin. Same type of resistant profile was also observed for General Hospital, Numan and General Hospital, Ganye.

A high percentage of isolates collected from Specialist Hospital, Yola showed resistance to antibiotics compared to that of General Hospital Mubi, General Hospital, Numan and General Hospital Ganye. The results were expressed in Table 4. At $p < 0.05$ level of significance, the chi-square calculated value was 216.34, which is far higher than the tabulated value of 53.38, therefore the null hypothesis is rejected, and alternate hypothesis accepted. The susceptibility of the *E. coli* to antibacterial agents routinely used in the hospitals as shown in Table 5 are similar those described for *S. aureus* above. At $p < 0.05$ level of significance, the chi-square calculated value was 231.81, which is far higher than the tabulated value of 53.38, therefore the null hypothesis is rejected, and alternate hypothesis accepted.

DISCUSSION

The presence of some pathogenic microorganisms causing hospital acquired infections and their

Table 2. Distribution of the isolates according to infectious sites in Adamawa State Hospitals.

Organism	Infection site												Hospital	
	Surgical wound		Catheter		Blood		UT		RT		SST			Total (%)
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)		
<i>E. coli</i>	11(8.66)	27.5	7(7.0)	17.5	6(5.33)	15	11(10.66)	27.5	2(3.0)	5	3(5.33)	7.5	40(33.3)	SHY
<i>S. aureus</i>	10(8.66)	25	6(7.0)	15	4(5.33)	10	5(10.66)	12.5	5(3.0)	12.5	10(5.33)	25	40(33.3)	
<i>C. albicans</i>	5(8.66)	12.5	8(7.0)	20	6(5.33)	15	16(10.66)	40	2(3.0)	5	3(5.33)	7.5	40(33.3)	
Total	26		21		16		32		9		16		120	
<i>E. coli</i>	12(9.0)	30	6(7.0)	15	3(4.33)	7.5	13(9.33)	35.5	23.67	5	4(6.67)	10	40(33.3)	GHM
<i>S. aureus</i>	9(9.0)	22.5	7(7.0)	17.5	5(4.33)	12.5	3(9.33)	7.5	4(3.67)	10	12(6.67)	30	40(33.3)	
<i>C. albicans</i>	6(9.0)	15	8(7.0)	20	5(4.33)	12.5	12(9.33)	30	5(3.67)	12.5	4(6.67)	10	40(33.3)	
Total	27		21		13		28		11		20		120	
<i>E. coli</i>	9(7.0)	22.5	8(7.33)	20	4(6.33)	10	9(8.33)	22.5	3(3.66)	7.5	7(7.33)	17.5	40(33.3)	GHN
<i>S. aureus</i>	8(7.0)	22.5	5(7.3)	12.5	7(6.33)	17.5	2(8.33)	5	6(3.66)	15	12(7.33)	30	40(33.3)	
<i>C. albicans</i>	4(7.0)	10	9(7.33)	22.5	8(6.33)	20	14(8.33)	35	2(3.66)	5	3(7.33)	7.5	40(33.3)	
Total	21		22		19		25		11		22		120	
<i>E. coli</i>	10(7.0)	25	2(8.66)	17.5	4(4.0)	10	15(12.0)	37.5	1(3.33)	2.5	3(5.00)	7.5	40(33.3)	GHG
<i>S. aureus</i>	8(7.00)	20	9(8.66)	25	4(4.00)	10	4(12.0)	10	5(3.33)	12.5	10(5.00)	25	40(33.3)	
<i>C. albicans</i>	3(7.00)	7.5	10(8.66)	25	4(4.0)	10	17(12.0)	42.5	4(3.33)	10	2(5.00)	25	40(33.3)	
Total	21		26		12		36		10		15		120	

Figures in brackets are expected values. SHY = Specialist Hospital, Yola, GHM = General Hospital, Mubi, GHN = General Hospital, Numan, GHG = General Hospital, Ganye, UT = Urinary tract, RT = Respiratory tract, SST = Skin and soft tissue.

susceptibility to antimicrobial drugs routinely used in four large hospitals in Adamawa State, Nigeria, have been investigated for a period of two and half years. Such large urban-based hospitals have been the centers of many investigations by researchers such as Pota schmacher et al. (1979), Regev-Yachan et al. (2001), Lark et al. (2001), Schroeder et al. (2002) and Bratu et al. (2005). Though from each hospital, 160 isolates of each of *S. aureus*, *C. albicans* and *E. coli* were collected, there were some variations in the proportion of isolates collected per site as well as between hospitals and these variations were statistically significant at $p < 0.05$ as depicted in Table 1. Some sites provided more isolates than

others. Such variations in the proportion of isolates within hospitals have previously been observed in a number of countries (Regev-Yachan et al., 2001; Lark et al., 2001; Tiemersma et al., 2004; Trick et al., 2004; Abu-Hajier and Sharif, 2008). Variation in the proportion and virulence of isolates between hospitals in the same country has also been reported by a number of scholars (Voss et al., 1994; Tortorano et al., 2002; Hope et al., 2002; Monnet et al., 2004). These variations may be due to differences in infectious control measures such as application of hygiene protocols to prevent transmissions, level of care needed by patients and antimicrobial prescription policies. Prevalence of HIV, cancer,

diabetics, variations in health care delivery and clinical practices, including the frequency of using blood cultures in diagnostics, antibiotic use patterns and resistance situations have been attributed to these differences in the proportion and virulence of isolates amongst sites and hospitals (Poikonen et al., 2003).

A significant proportion of isolates in this study were from specimens obtained from urinary tract sites. This is because soon after admission, microorganisms prevalent in the hospital environment colonize the patient's skin, gastrointestinal tract and the anterior urethra. With the insertion of catheter, the microbes may be causing hospital acquired infections and their susceptibility to

Table 3. Antibacterial resistance profile of *C. albicans* isolates in Adamawa state hospitals.

Antifungal drug	Hospital								
	SHYola		GH Mubi		GH Numan		GH Ganye		Total
	N	(%)	N	(%)	N	(%)	N	(%)	N(%)
Nystatin	12(11.17)	30	7(9.75)	17.5	10(10.56)	25	11(8.53)	27.5	40
Itraconazole	11(12.00)	27.5	10(10.48)	25	12(11.35)	30	10(9.17)	25	43
Miconazole	13(11.44)	32.5	11(9.9)	27.5	9(10.82)	22.5	8(8.74)	20	41
Fluconazole	10(11.17)	25	12(9.75)	30	10(8.71)	27.5	7(8.53)	17.5	40
Ketoconazole	9(9.21)	22.5	8(8.04)	20	10(8.71)	25	6(7.03)	15	33
Total	55		48		52		42		197

$\chi^2 = 69.65$; $df = 12$ and tabulated value = 21.03 Figures in brackets are expected values, SHY = Specialist Hospital Yola, GHM = General Hospital, Mubi, GHN = General Hospital Numan, GHG = General Hospital Ganye.

Table 4. Antibacterial resistance profile of *S. aureus* isolates in Adamawa state hospitals.

Antibacterial drug	Hospital								
	SHYola		GH Mubi		GH Numan		GH Ganye		Total
	N	(%)	N	(%)	N	(%)	N	(%)	N (%)
Ciproflox	11(12.63)	27.5	12(11.62)	30	10(10.38)	25	11(9.37)	35	44(5.6)
Norfloxacin	9(8.90)	22.5	7(8.18)	17.5	7(7.32)	17.5	8(6.60)	40	31(4.0)
Gentamycin	17(15.50)	42.5	11(14.26)	27.5	14(12.74)	35	12(11.50)	25	54(6.9)
Lincocin	14(12.05)	35	13(11.09)	32.5	8(9.91)	20.5	7(8.95)	32.5	42(5.4)
Streptomycin	16(14.35)	40	13(13.20)	32.5	11(11.80)	27.5	10(10.65)	7.5	50(6.3)
Rifampicin	10(12.05)	25	11(11.09)	27.5	13(9.91)	32.5	8(10.65)	65	42(5.4)
Floxapen	7(9.47)	17.5	10(8.71)	25	11(7.79)	27.5	5(7.03)	37.5	33(4.2)
Erythromycin	15(12.05)	37.5	9(11.09)	22.5	10(9.91)	25	8(10.65)	42.5	42(5.4)
Chloramphenicol	12(15.50)	30	15(14.26)	37.5	13(12.74)	32.5	14(11.50)	7.5	54(6.9)
Ampiclox	13(12.63)	32.5	12(11.62)	30	10(10.38)	25	9(9.37)	17.5	44(5.6)
Cloxacillin	15(16.65)	37.5	22(15.31)	30	11(13.69)	27.5	10(12.35)	15	58(7.3)
Tetracycline	29(29.8)	72.5	27(27.46)	67.5	26(24.54)	65	22(22.15)	12.5	104(13.3)
Penicillin	26(24.1)	65	21(22.18)	52.5	20(19.82)	50	17(17.89)	60	84(10.7)
Ampicillin	31(29.27)	77.5	24(26.93)	60	21(24.07)	52.5	26(21.73)	45	102(13.0)
Total	225		207		185		167		784

$\chi^2 = 216.34$; $df = 39$ and tabulated value = 53.38. Figures in brackets are expected values, SHY = Specialist Hospital Yola, GHM = General Hospital, Mubi, GHN = General Hospital Numan, GHG = General Hospital Ganye.

antimicrobial drugs routinely used in four large hospitals in Adamawa State, Nigeria, have been investigated for a period of two and half years. Such large urban-based hospitals have been the centers of many, some patients may have a greater chance of becoming colonized or infected with these bacteria and these are similar to the observations of Fluit et al. (2001) and Abu-Hajier and Sharif (2008). The explanation of high occurrence of the bacteria in surgical units could be due to the long periods of stay in these units and the extensive use of broad spectrum antibiotics. The results in Table 2 showed that *E. coli* was a major cause of infections affecting the catheter, surgical wound, blood and urinary tract sites. *E. coli* is one of the most common cause of nosocomial infections by gram-negative bacilli and the most common bacteria isolated from surgical wound and blood sites

(Stelling et al., 2005). This organism has been implicated in causing such infections at these sites especially among the immunocompromised and the critically ill patients (Lark et al., 2001; Oteo et al., 2005). These organisms colonize the lower digestive tract and cause infections in patients operated on lower abdominal surgery. Strains of the bacterium have also been isolated in the respiratory tract, particularly in gastric tube fed patients, burns and soft tissue sites in this study, just like in earlier studies (Johnson et al., 2001; Schroeder et al., 2002). The *E. coli* strains identified in this study were non-O157 serotypes- 026, 091, 0103, 0111, 0128 and 0145 which are mostly associated with verocytotoxin production. The proportion of *E. coli* isolated was 27.5% from surgical wound and urinary tract sites, 17.5% from catheter sites, 15% from blood samples, 7.5% from skin

Table 5. Antibacterial resistance profile of *E. coli* isolates in Adamawa State Hospitals.

Antibacterial drug	Hospital								Total N (%)
	SH Yola		GH Mubi		GH Numan		GH Ganye		
	N	(%)	N	(%)	N	(%)	N	(%)	
Nitrofurantoin	13(14.11)	32.5	11(12.72)	27.5	13(12.45)	32.5	14(11.72)	35	51(6.6)
Gentamycin	20(19.05)	50	16(17.20)	40	17(16.85)	42.5	16(10.48)	40	69(10.0)
Nalidixic acid	10(14.94)	37.5	18(13.46)	45	16(13.18)	35	10(12.41)	25	54(7.0)
Ofloxacin	10(11.62)	25	12(10.47)	30	7(10.25)	17.5	13(9.65)	32.5	42(5.5)
Augmentin	8(6.08)	20	6(5.49)	15	5(5.37)	12.5	3(5.06)	7.5	22(2.9)
Tetracycline	33(32.36)	82.5	30(29.17)	75	28(28.57)	7	26(17.78)	65	117(15.2)
Amoxicillin	12(21.30)	30	11(13.96)	27.5	18(13.67)	45	15(12.87)	37.5	56(7.3)
Co-trimoxazole	22(15.30)	55	18(19.20)	45	20(18.80)	50	17(17.70)	42.5	77(10.0)
Tarivid	6(5.26)	15	4(4.74)	10	6(4.64)	15	3(4.37)	7.5	19(2.5)
Peflocine	8(7.19)	20	5(6.48)	12.5	6(6.35)	15	7(5.98)	17.5	26(3.4)
Ciporex	10(7.47)	25	6(6.73)	15	5(6.59)	12.5	6(6.20)	15	27(3.5)
Ciproflox	9(6.92)	22.5	7(6.23)	17.5	4(6.10)	10	5(5.75)	12.5	25(3.3)
Ampicillin	33(31.26)	80	29(28.18)	72.5	27(27.59)	67.5	24(17.17)	60	113(14.7)
Streptomycin	19(19.92)	47.5	19(17.95)	47.5	16(17.58)	40	18(16.55)	45	72(9.4)
Total	213		192		188		177		770

$\chi^2 = 231.81$; $df = 39$ and tabulated value = 53.38. Figures in brackets are expected values, SHY = Specialist Hospital Yola, GHM = General Hospital, Mubi, GHN = General Hospital Numan, GHG = General Hospital Ganye.

and soft tissue sites and 5% from respiratory tract sites in the Specialist Hospital, Yola, and these trends are similar in the other hospitals. A significant proportion of the *S. aureus* have also been isolated from surgical wound, catheter, blood stream, respiratory and urinary tract sites as shown in Table 2 and these compared very well with those obtained elsewhere (Tambic et al., 1999; Tenover and Pearson, 2004; Li et al., 2005, Nguyen et al., 2005, Bratu et al., 2005). Many reports of nosocomial *S. aureus* infections have described strains harbouring the panton-valentine leukocidin determinant, a virulence factor for skin and respiratory tract infections particularly pneumonia (Mulvery et al., 2005).

Data obtained in this study showed that *C. albicans* was the most common organism associated with urinary tract (30 - 42.5%) and catheter site infections (20 - 25%) in these selected hospitals as shown in Table 2. It also caused significant blood, respiratory tract and skin and soft tissue infections. *Candida* is known to colonize indwelling devices, such as joint prostheses, prosthetic heart valves and central nervous and urinary catheters (O' Gara and Humphrey, 2001; Kuhn et al., 2004) and these may explain the predominance of the organism in urinary tract and catheter sites obtained in this study. Most patients infected with *C. albicans* in their catheters were observed to progress into blood streams infection (Saiman et al., 2002; Kuhn et al., 2004). In this study, some *C. albicans* have been isolated from preterm neonates and the severely ill patients similar to the reports obtained from other studies (Kao et al., 1999; Levy et al., 2001; Saiman et al., 2002). The isolation of *Candida* sp. from neonates especially those who have

low birth weight and who have undergone surgery, have been associated with parenteral nutrition and central lines (Levy et al., 2001; Kao et al., 1999). Biofilm formation on catheter and other devices and colonized surfaces is a virulence factor amongst the invasive strains *C. albicans* (Kuhn et al., 2004), the detachment of which can result in septicemia (Chandra et al., 2001).

In this study, a significant number of isolates of *C. albicans* obtained from hospitalized patients in Specialist Hospital, Yola were moderately resistant to miconazole (32.5%), nystatin (30%) and itraconazole (27.5%) as shown in Table 3. The antimicrobial sensitivities varied among the hospitals included in the study and the differences were statistically at $p < 0.05$ level of significance. Similar observations have been reported in some Indian hospitals (Mohanti et al., 2005). Microorganisms and their resistance patterns vary from hospital to hospital and even from clinic to clinic in the same hospital (Snydman, 1991; Savas et al., 2006). Differences in resistance rates can be of a particular significance because the spread of resistant strains from one country can influence susceptibility rates in other countries (Sahm et al., 2008). The results from the susceptibility testing showed that the Specialist Hospital, Yola had the highest resistance rates for the organisms followed by the General Hospital Mubi, General Hospital Numan and General Hospital Ganye and these report agreed with that of Jamort et al. (2005) in Japan, United States and Europe as well as that of Abu-Hajier and Sharif (2008) in Palestine. In all these countries, the resistance rates were reported to be higher in the principal referral metropolitan hospitals than in general metropolitan

hospitals and the smaller private hospitals. The susceptibility data obtained in this study as depicted in Table 4, showed that *S. aureus* was resistant to penicillin (65%), ampicillin (77.5%) and these compared very well with data obtained in other countries (Tambic et al., 1999; Regev-Yochan et al., 2005; Collignon et al., 2005; Abu-Hajier and Sharif, 2008). Resistance of *E. coli* to ampicillin (80%) and tetracycline (82.5%) were particularly high in the Specialist Hospital, Yola than the other three hospitals as shown in Table 5. High-level resistance was recorded in Specialist Hospital, Yola, where a combination of severely ill patients, invasive technologies and high level use of antimicrobial agents facilitates the selection, emergence and spread of multi-drug resistant pathogens similar to the reports of Abu-Hajier and Sharif (2008) in Palestine. All the isolates were moderately susceptible to tarivid as well as the quinolone antibiotics, similar to the reports of El-Mahmood et al. (2009). Apart from the volume of use of an antibiotic, horizontal gene transfer, cross-transmission between patients, acquisition of microorganisms from the hospital environment, are all factors that influence the emergence and spread of antibiotic resistance within the hospital setting (Tambic et al., 1999; Brown et al., 2005). All the isolates in this study showed resistance to at least 1 - 3 different antimicrobial drugs, indicating the presence of strong selective pressures from the antibiotics in the hospitals. The results showed that a significant number of *S. aureus* and *E. coli* isolates obtained in Specialist Hospital, Yola were resistant to the older antibiotics, ampicillin, tetracycline, co-trimoxazole, gentamycin, but moderately sensitive to tarivid and fluoroquinolone antibiotics.

We are unable to distinguish the different mechanisms of resistance, or to determine the presence of any clone responsible for the high endemic levels of antimicrobial resistance in the four large hospitals. Nevertheless, the results can serve to direct any rational effort aimed towards reducing nosocomial infections and antimicrobial resistance problems in these large referral hospitals as well as other local hospitals in the State.

REFERENCES

- Abu-Hajier AS, Sharif FA (2008). Detection of methicillin-resistant *Staphylococcus aureus* in nosocomial infection in Gaza Strip. *Afr. J. Microbiol. Res.*, 2: 235-241
- Archibald LK, Tuohy MJ, Wilson DA, Nwanyauwu O, Kazambe PN, Tansuphasawadikul S, Eanpokalap B, Chaovolnich A, Reller LB, Jarvis WR, Hall GS, Procop GW (2004). Antifungal susceptibilities of *Cryptococcus neoformans*. *Emerg. Infect. Dis.* 10(1):143-145.
- Adamawa State Statistical Year Book (2000). In: Medical and Health Statistics. Adamawa State Planning Commission (publishers). 2: 90-122.
- Amita S, Chowdhury R, Thungapathia M, Ramamuthy T, Nair GB, Ghosh A (2003). Class1 Integrins and SXT Elements in EL Tor Strains isolated before and after 1992 *Vibrio cholerae* O139 outbreak, Calcutta, India. *Emerg. Infect. Dis.* 9(4): 500-502.
- Beaudin BA, Brosnikoff CA, Grimsrud KM, Heffner TM, Rennie RP, Talbot JA (2004). Susceptibility of human isolates of *Salmonella typhimurium* DT104 to antimicrobial agents used in human and veterinary medicine. *Diagn. Microbiol. Infect. Dis.*, 42:7-20.
- Bauer AW, Kirby WM, Sharris JC, Jurck M (1966). Antibiotic susceptibility testing by a standard single disk method. *Am. J. Clin. Pathol.*, 45: 493-496
- Bratu S, Eramo A, Kopec R, Coughlin E, Ghitan M, Yost R, Chapnick EK, Landman D, Quale J (2005). Community-associated Methicillin-resistant *S. aureus* in Hospital, Nursing and Maternity Units. *Emerg. Infect. Dis.*, 11(6): 808-812.
- Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgeway DL, Towner K J (2005). Guidelines for the laboratory diagnosis and susceptibility testing of methicillin resistant *Staphylococcus aureus* (MRSA). *J. Antimicrob. Chemother.*, 56(6): 1000-1018.
- Chandra J, Kuhn DM, Mulhejee PK, Hoyer LL, McCormick T, Ghannoum MA (2001). Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *J. Appl. Bacteriol.*, 183(5): 385-5394
- Cheesbrough M (2002). Microbiological tests. In "District Laboratory Practice in Tropical Countries", part 2. (Cheesbrough, M edn). The Cambridge University press. pp. 1-487.
- Clinical and Laboratory Standard Institute (2006). Zone diameter Interpretive Standards and corresponding minimal inhibitory concentration (MIC) interpretive break points. Supplement M44:S1. Clinical and Laboratory Standard Institute, Wayne, PA
- Collignon P, Nimma GR, Gottlieb T, Gosbell IB (2005). *Staphylococcus aureus* Bacteremia, Australia. *Emerg. Infect. Dis.* 11(4): 554-561.
- El-Mahmood AM, Atimi AT, Tirmidhi AB, Mohammed A (2009). Antimicrobial susceptibility of some quinolone antibiotics against some urinary tract pathogens in a tertiary hospital, Yola, Adamawa State, Nigeria. *J. Clin. Med. Res.*, 1(2): 26-34.
- Fluit AC, Maarten R, Visser A, Schmidz FJ (2001). Molecular detection of Antimicrobial Resistance. *Clin. Microbiol. Rev.*, 14: 836-871
- Gupta A, Nelson JM, Baret T, Tauxe RV, Rossiter SP, Friedman CR, Joyce KW, Smith KE, Jones TF, Hawkins MA, Shiferaw B, Beebe JL, Vugia DJ, Rabatsky-Ehr T, Benson-Root JP, Angulo FJ (2004). Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. *Emerg. Infect. Dis.*, 10(6): 1102-1109.
- Hope W, Morton A, Eisen DP (2002) Increase in prevalence of nosocomial non-*Candida albicans* candidemia and the association of *Candida krusei* with fluconazole use. *J. Hosp. Infect.*, 50: 499-511.
- Jamort S, Denis O, Deplano A, Tragas G, Vanderghyest A, Bels DD, Devriendt J (2005). Methicillin-resistant *S. aureus* Toxic Shock Syndrome. *Emerg. Infect. Dis.*, 11(6): 366-370.
- Johnson JR, Delavari P, Kuskowski M, Stell AL (2001) Phylogenetic distribution of extraintestinal virulence-associated traits in *E. coli*. *J. Infect. Dis.*, 183: 78-88.
- Kao PA, Chin OC, Mangwan FA (1999). Inducible clindamycin resistance in staphylococci: should clinicians and microbiologists be concerned. *Clin. Infect. Dis.* 40: 280-285
- Kuhn DM, Mukherjee PK, Clrak TA, Pujol C, Chandra J, Hajjeli RA, Warnock DW, Soll DR, Ghannoum MA (2004) *Candida parapsilosis* characterization in an outbreak setting. *Emerg. Infect. Dis.* 10(6): 1074-1081.
- Lark RL, Saint S, Chenoweth, C, Zemencuk JK, Lipsky BA, Plorde JJ (2001). Four-year prospective evaluation of community-acquired bacteremia: Epidemiology, Microbiology, and Patient Outcome. *Diagn. Microbiol. Infect. Dis.*, 41:15-22.
- Lark SK (2001). Community associated methicillin-resistant *Staphylococcus aureus* and its emerging virulence. *Clin. Med. Res.* 3: 5-60
- Lesch CA, Hokazu GS, Danziger LH, Robert A, Weinslein A (2001). Multi-hospital analysis of antimicrobial usage and resistance trends. *Diagn Microbiol. Infect. Dis.*, 41: 149-154.
- Levy PA, Okai LM, Manger PA (2001). Guidelines for Isolation precautions in hospitals. *Infect. Contr.*, 4: 245-325.
- Li F, Park SY, Ayers TL, Miller FD, MacFadden R, Nakata M, Lee MC, Effler PV (2005). Methicillin-resistant *S. aureus*, Hawaii, 2000-2002. *Emerg. Infect. Dis.* 11(8): 1205-1210.
- Madigan MT, Martinko JM, Parker J (2000). Epidemiology and Public Health Microbiology. In: Brock Biology of Microorganism Madigan M.T. Martinko JM, Parker J. (eds), 9th edn Prentice Hall, pp. 841-955.
- Mohanti S, Singal R, Sood S, Dhawan B, Das BK, Kap A (2005). Comparative *in vitro* activity of beta-lactam/beta-lactamainhibitor

- combinations against Gram negative bacteria. *Indian J. Med. Res.*, 122: 425-428.
- Monnet D, MacKenzie FM, Lopez-Lozana JM, Beyaert A, Camacho M, Wilson R, Stuart D, Gould IM (2004). Antimicrobial drug use and methicillin-resistant *S. aureus*, Aberdeen, 1996-2000. *Emerg. Infect. Dis.* 10(8): 1432-1441.
- Mulvey MR, McDougall L, Cholin B, Horseman G, Fidyk M, Woods S (2005). Community-associated methicillin-resistant *S. aureus*, Canada. *Emerg. Infect. Dis.* 11(6): 844-850.
- Nguyen DM, Mascola L, Bancroft E (2005). Recurring Methicillin-resistant *S. aureus* Infections in a football team. *Emerg. Infect. Dis.* 11(4): 526-532.
- Nkang AO, Okonko IO, Fowotade A, Udeze AO, Ogunnusi AT, Fajobi EA, Adewale OG, Mejeha OK (2009). Antibiotics susceptibility profiles of bacteria from clinical samples in Calabar, Nigeria. *J. Bacteriol. Res.*, 1(8): 89-96.
- O'Gara JB, Humphreys H (2001). *Staphylococci epidermidis* biofilms: importance and implications. *J. Med. Microbiol.*, 50: 582-587.
- Oteo J, Lazaro E, Abajo FJ, Baquero F, Campos J (2005). Antimicrobial-resistant invasive *E. coli*, Spain. *Emerg. Infect. Dis.*, 11(4): 546-553.
- Pfaller MA, Jones RN, Biedenbach A (2001). Antimicrobial resistance trends in Medical Centres using carbapenems, report of 1999 and 2000 results from the MYSTIC Programme (USA). *Diagn. Microbiol. Infect. Dis.*, 41: 177-182.
- Pfaller MA, Diekema DJ, Sheehan DJ (2006). Interpretive breakpoints for fluconazole and candida revisited: a blueprint for the future of antifungal susceptibility testing. *Clin. Microbiol. Rev.*, 19: 435-447.
- Pfaller MA, Messer SA, Boiken L, Rice C, Tendolkr S, Nollis RJ, Doern GV, Diekema DJ (2005). Global trends in the antifungal susceptibility of *Cryptococcus neoformans* (1990-2004). *J. Clin. Microbiol.*, 43: 2163-2167.
- Poikonen E, Lyytikäinen O, Anttila VJ, Runtu P (2003). Candidemia in Finland, 1995-1999. *Emerg. Infect. Dis.*, 9(8): 985-990.
- Potaschmacher LO, Dash CH, Jefferson KA, Kennedy MR (1979). A survey of the sensitivity of fresh clinical isolates to cefuroxime and other antibiotics. *J. Clin. Pathol.*, 32(9): 944-950.
- Regev-Yachan D, Rubinstein E, Barzilai A, Carmeli Y, Kuint J, Etienne J, Blech M, Smollen G, Maayan-Metzger J, Leavitt A, Rahav G, Keller N (2005). Methicillin-resistant *S. aureus* in Neonatal Intensive Care Unit. *Emerg. Infect. Dis.*, 11(3): 453-456.
- Saiman L, Ludington E, Pfaller M, Rangel-Frausto S, Wiblin RT, Dawson J (2002). Risk factors for candidemia in Neonatal Intensive Care Unit patients. *Paediat. Infect. Dis. J.*, 19: 319-324.
- Savas L, Guvel S, Onlen Y, Savas N, Duran N (2006). Nosocomial urinary tract infections: microorganisms, antibiotic sensitivities and risk factors. *West Indian Med. J.*, 55(3): 1-9
- Sherer CR, Sprague BM, Campos JM, Nambiar S, Temple R, Short B, Singh N (2005). Characterizing vancomycin-resistant Enterococci in Neonatal Intensive Care Unit. *Emerg. Infect. Dis.*, 11(9): 1470-1474.
- Schroeder CM, Meng J, Zhao S, Debroy C, Torcolini J, Zhao C, McDermott PF, Wagner DD, Walker RD, White DG (2002). Antimicrobial resistance of *Escherichia coli* 026,0103,0111,0128 and 0145 from animals and humans. *Emerg. Infect. Dis.*, 8(12): 1409-1414.
- Snydman DR (1991). Clinical implications of multi-drug resistance in the intensive care unit. *Scan J. Infect. Dis.*, 78: 54-63
- Stelling JM, Travers K, Jones RN, Turner PJ, O'Brien TF, Levy SB (2005). Integrating *E. coli* Antimicrobial Susceptibility Data from Multiple Surveillance Programmes. *Emerg. Infect. Dis.*, 11(6): 873-882.
- Tambic A, Power EG, Tambic T, Snur I, French GL (1999). Epidemiological analysis of methicillin-resistant *Staphylococcus aureus* in a Zagreb Trauma Hospital using a randomly amplified polymorphic DNA-typing method. *Eur. J. Clin. Microbiol. Infect. Dis.*, 18: 335-340.
- Tenover FC, Pearson M (2004). Methicillin-resistant *S. aureus*. *Emerg. Infect. Dis.*, 10(11): 2052-2053.
- Tiemersma EW, Bonzwaer SLAM, Lyytikäinen O, Degener JE, Schrijnemakers P, Bruinsma N, Monen J, Witte W, Grundmann H (2004). Methicillin-resistant *S. aureus* in Europe, 1999-2002. *Emerg. Infect. Dis.*, 10(4): 1627-1634.
- Tortorano AM, Biraghi E, Astolfi A, Ossi C, Tejada M, Farina C (2002). European Confederation of Medical Mycology (ECMM). Prospective survey of candidemia: report from one Italian region. *J. Hosp. Infect.*, 51: 297-304.
- Trick WE, Zargoski BM, Tokars JL, Vernon MO, Welbel SF, Wisniewski MF, Richards C, Weisstein RA (2004). Computer algorithms to detect blood stream infections. *Emerg. Infect. Dis.*, 10(9): 1612-1620.
- Voss A, Milatoric D, Waelrauch-schwarz C, Rosdahl VT, Braveny L (1994). Methicillin-resistant *S. aureus* in Europe. *Eur. J. Microbiol. Infect. Dis.* 13: 50-55.