Published Quarterly
Mangalore, South India
ISSN 0972-5997
Volume 6, Issue 1; Jan -Mar 2007

Original Article

Chromobacterium violaceum associated with recurrent vaginal discharge among apparently healthy females in Ekpoma, Nigeria

Authors
Agwu Ezera
Department of Medical Microbiology, College of Medicine, Ambrose Alli University, Box 14, Ekpoma, Edo State, Nigeria
Ihongbe John Cletus
Department of Medical Laboratory Sciences, College of Medicine, Ambrose Alli University, Box 14, Ekpoma, Edo State, Nigeria
Okogun Godwin Ray Anugboba
Department of Medical Laboratory Sciences, College of Medicine, Ambrose Alli University, Box 14, Ekpoma, Edo State, Nigeria
Ezeonwumelu Joseph Obiezu Chukwujekwu
Department of Pharmacy, Clinical Division, Nigerian Institute of Medical Research, 6, Edmond Crescent, PMB, 2013, Yaba, Lagos, Nigeria
Igbinovia Osamuyime
Department of Medical Microbiology, College of Medicine, Ambrose Alli University, Box 14, Ekpoma, Edo State, Nigeria

Address For Correspondence
Agwu Ezera
Department of Microbiology,
School of Health Sciences, Kampala International University,
Western Campus, Box 71,
Bushenyi District, Uganda.
E-mail: agwuezera@yahoo.com

Citation
Agwu E, Ihongbe JC, Okugun GRA, Ezeonwumelu JOC, Igbinovia O. *Chromobacterium violaceum* associated with recurrent vaginal discharge among apparently healthy females in Ekpoma, Nigeria *Online J Health Allied Scs.* 2007;1:2

URL
http://www.ojhas.org/issue21/2007-1-2.htm

Open Access Archives
http://cogprints.ecs.soton.ac.uk/view/subjects/OJHAS.html
http://openmed.nic.in

Submitted Oct 24, 2006; Accepted: June 18, 2007; Published: July 17, 2007

Abstract:
Seven hundred and two high vaginal swab samples of apparently healthy adult females with recurrent vaginal discharge were collected and analyzed at Searchlight Medical Diagnostic Centre, Ekpoma between July 2003 and August 2005. Isolation, identification and antibiogram of isolates were done using standard Microbiological techniques. Randomized Block Design and Completely Randomized Design at ($\alpha = 0.1$) were used to test the statistical significance of results. While occupational distribution of isolates was not statistically significant, age and response of bacterial isolates to antibiotics used were statistically significant. Total number and percentage prevalence of bacteria isolated include: 297(42.4%) *Chromobacterium violaceum,* 156 (22.2%) *Escherichia coli* and 139 (19.8%) *Staphylococcus aureus*. *Chromobacterium violaceum* was 81.8% sensitive to ofloxacin, and 12.8% to Ceftriazone. *Escherichia coli* and *S. aureus* were 70.5% and 71.9% sensitive to sparfloxacin respectively. Though source of infection of *C. violaceum* in Ekpoma was not very clear, soil and water contamination and other risk factors were hypothesized. A high index of suspicion is required for diagnosis. Surveillance with improved diagnostic facilities can increase awareness among health care providers on this form of infection. Appropriate systemic antimicrobial therapy to halt progression of infection is mandatory, even when the infection appears to be localized.
Key Words: *Chromobacterium violaceum,* Recurrent vaginal discharge, Ekpoma

Introduction:
The rising episodes of recurrent vaginal discharge among apparently healthy females are increasingly becoming a cause for concern for health care providers in Ekpoma. Vaginal discharge may be due to infection of the vagina, cervix or uterus. Three types of vaginal discharge which had been described[1] include: purulent vaginal discharge attributed to *Trichomonas vaginalis*, white odourless discharge attributed to *Candida albicans,* and a thin grayish-white discharge with a characteristic ammoniacal fishy odour attributed to *Gardnerella vaginalis*.

Chromobacterium violaceum, a saprophytic bacterium, was first discovered in water buffalo by Wooley in 1905.[2] It is unique to tropical and subtropical climates and is found between latitudes 35^0N and 35^0S. This includes Malaysia, where this facultative anaerobic gram-negative rod bacterium was first seen in humans in 1927.[3] It has also been isolated from soil and water from Trinidad, Brazil, India, Malaysia, Thailand and Vietnam. There have been fewer than 40 cases of human infection reported, and several have come from the southeastern United States, primarily Florida.[4] The organism is a well-known inhabitant of soil and water - particularly stagnant or slow-moving water sources. An underlying defect in host defense, especially that of neutrophils, seems to predispose to infection and of the cases reported in the United States, 73% have ended in death.[5] Most reported infection by *Chromobacterium violaceum* is associated with bacteremia but very little genitor-urinary infection has also been reported though not in sub-Saharan Africa.[6]

Vaginal discharge cases in Ekpoma are still being treated empirically with the assumption that their cause might either be *Trichomonas vaginalis, Candida albicans* or *Gardnerella vaginalis.*[7] Due to high index of suspicion of involvement of other microbes in recurrent vaginal discharge, this investigation was therefore designed to ascertain if other microbes (*Chromobacterium violaceum*) apart from *Trichomonas vaginalis, Candida albicans* or *Gardnerella vaginalis* may be involved in the rising episodes of recurrent vaginal discharge among females in Ekpo-

ojhas Online Journal of Health and Allied Sciences

ma Nigeria. This investigation was also aimed at outlining the susceptibility profile of *C. violaceum,* and other bacteria isolates identified.

Materials and Methods:

Sampling Area

Ekpoma, the main study area, is a University town situated 120km north of Benin, the capital city of Edo State, Nigeria. It has few private clinics with no specialist or referral hospital. All referral cases are usually sent to Irrua Teaching Hospital located in a nearby community.

Ethical considerations

Informed consent on this investigation was sought and obtained verbally from each subject and in writing from the ethics committee of SMDC, before commencement of this study. Those who could not read or write in English language gave their verbal approval after the aims and benefits of this investigation were explained to them in their local language.

Sample collection and inclusion criteria

Seven hundred and two (702) High Vaginal Swab (HVS) samples of apparently healthy adult females with recurrent vaginal discharge were collected and analyzed at Searchlight Medical Diagnostic Centre (SMDC), Ekpoma over a two year period between July 2003 and August 2005. All samples were collected with a sterile swab stick and with the aid of a sterile vaginal speculum used to bypass the vagina to get a HVS sample. The nature of the discharge ranged from yellowish green purulent, white odourless, and greyish offensive smelling discharges respectively, and also their pH ranged from 5 to 6.5

Subjects only qualified for inclusion in this investigation if: they had Recurrent Vaginal Discharge (RVD) within 1 week after therapy, their condition appeared ineffective for local antimicrobial chemotherapy (e.g. with metronidazole, cotrimoxazole, gentamicin etc), vaginal pH ranged between 5 & 6.5, and came to Searchlight Medical Diagnostic Centre (SMDC), Ekpoma for investigation on their own or were referred. Recurrent Vaginal Discharge (RVD), used in this context refers to a vaginal discharge that had persisted after presumptive treatment with different antibiotics (metronidazole, cotrimoxazole, gentamycine etc) without laboratory investigation.

Media preparation and cultivation of samples

MacConkey agar, Blood agar base, Sabouraud Dextrose Agar (SDA), Nutrient agar and Mueller Hinton sensitivity testing agar medium were prepared according to manufactures (OXOID Limited UK) instructions. They were sterilized at 121^0C for 15 minutes holding time in an autoclave. Ten percent (10%) blood agar was prepared by mixing 10 ml fresh sheep blood with 90 ml molten blood agar base at about 45^0C. About 20 ml of each medium was dispensed on a sterile deposable plastic petri dish and allowed to set.

Samples were inoculated unto MacConkey, Nutrient, and Blood agar plates respectively for bacterial isolation and unto SDA plates for fugal isolation. They were all incubated at 37 ± 3^0C for 18-24 hours. Direct gram smear were made on a microscope slide and wet mounts prepared and examined microscopically.

Analysis of isolates

All suspect colonies for *Chromobacterium violaceum*, showing variable degree of partial hemolysis on blood agar and plentiful violet pigmented colonies on both Nutrient and MacConkey agar were picked for further analysis. Other suspect medically important bacteria colonies on the plates were also picked

for further analysis. All suspect colonies for *Chromobacterium violaceum* were identified according to the scheme of Cowan and Steel's Manual for the identification of medically important bacteria as revised by Barrow and Feltham.[8] Significant mixed growth of bacteria colonies (more than 25 colonies per plate) were separated into single colonies by obtaining purity plates. The test conducted for bacterial identification included: Direct and indirect gram staining, oxidase, catalase, coagulase tests, urea fermentation, citrate utilization, and sugar fermentation test with lactose, sucrose, D-mannitol, D-sorbitol, glucose, fructose, mannose, and trehalose. The tests were performed under aseptic conditions following the procedures previously described by Cheesbrough.[9] Germ tube test and chlamydospore test were done to confirm isolates as *Candida albicans* following the steps outline by Raphael.[10]

Antibiotic susceptibility test

Commercially prepared antibiotic disc (Difco) was used. Kirby-Bauer's National Committee for Clinical Laboratory Standards (NCCLS) modified disc diffusion technique for the antibiotic susceptibility test was adopted in this investigation.[11] Control of test performance outlined by Cheesbrough[9] was strictly followed. After incubation at 35^0C for 18-24 hours, zone sizes were measured and interpreted using NCCLS standards.[12,13]

The criterion for antibiotic inclusion was based on first line broad spectrum antibiotics commonly used in Ekpoma and in line with the guideline for antibiotic selection during susceptibility testing, by National Committee for Clinical Laboratory Standards (NCCLS).[13] The antibiotics included and their disc antibiotic contents were ciprofloxacin (5µg), Ofloxacin (5µg), sparfloxacin (5µg), gentamicin (10µg), ampicillin-cloxacillin (ampiclox) (10µg), cotrimoxazole (300µg), oxacillin (1µg), ceftriaxone (30µg), cefuroxime (30µg), rifampicin (5µg), and erythromycin (15µg).

Statistical Analysis

The Randomized Block Design (RBD) at (α = 0.1 or 99% level of confidence) summarized as [Total sum of squares (SST) = Sum of squares due to different effects (SST) + Error sum of effects (SST)][14], for one observation per treatment was used to test if occupation and age may be considered significant risk factors in the prevalence of isolates among the study population (Table 1 and 2). The Completely Randomized Design (CRD) at (α = 0.1 or 99% level of confidence) for unequal number of observations per treatment was used to test if the reaction of isolates to different antibiotic treatment differs significantly (Table 3).

Results

The result of the analysis of 702 HVS samples yielded a total of: 297(42.4%) *Chromobacterium violaceum* 156 (22.2%) *Escherichia coli* and 139 (19.8%) *Staphylococcus aureus* respectively. The distribution of isolates in single and in mixed infection is shown in (Table 4) below. The bacteria that showed the highest prevalence was *C. violaceum*, followed by *E coli.* No isolate yielded a growth of the 3 isolates with *Candida albicans*. There were 112 (16.0%) of *Candida albicans* and 31 (4.4%) *Tichomonas vaginalis* observed.

Table 1 depicts the occupational distribution of isolates. The prevalence of *C. violaceum* was highest among the group called "others" 65 (67.7%) which do not fit into any of the occupational classifications mentioned in (Table 1). The least was civil servants 5 (9.6%). Prevalence rate of *E. coli* was highest among students 63 (36.4%) and lowest among civil servants 0 (0.0%). *S. aureus* had the highest prevalence 55 (17.4%) while the group identified as others had 0 (0.0%) prevalence.

Using the randomized block design ($\alpha = 0.1$) there was no significant difference in the way isolates was distributed in relation to occupation

Table 2 shows the distribution of bacterial isolates in terms of age. The various age groups which yielded the highest bacterial isolates are as follows: 11-20 years had 60 (57.7%) *C. violaceum*, 31-40 years had 63 (25.8%) *E.coli*, and 41-50 had 14 (31.1) *S. aureus*.

Using the randomized block design at ($\alpha = 0.1$), there was a significant difference in the way isolates was distributed in relation to age group.

Table 3 depicts distribution of percentage susceptibility of isolates from 702 samples of females with recurrent vaginal discharge in Ekpoma. *C. violaceum* generally showed the highest sensitivity to the floro-quinolones with ofloxacin (81.8%) toping the list. *C. violaceum* was poorly susceptible to the cephalosporines used. *E coli* also showed the highest susceptibility to the floro-quinilones used in this investigation with sparfloxacin 110 (70.5) being on top. *S. aureus* which showed 100 (71.9%) sensitivity to sparfloxacin was the highest recorded among the antibiotics used for *S. aureus*. Summary of the rest of the sensitivity results obtained are shown in Table 3.

Using the completely randomized design ($\alpha = 0.1$) there was significant difference in the way isolates responded to different antibiotics used for treatment.

Table 1: Distribution of Isolates by occupation ([1]N= 702)

Occupation	Number examined	Number (%) positive for isolates		
		C. violaceum[2]	*E. coli*[3]	*S. aureus*[4]
Traders	136	70 (51.5)	22 (13.2)	16 (8.8)
Students	173	81 (46.8)	63 (36.4)	25 (7.5)
Teachers	73	12 (16.4)	9 (12.3)	10 (13.7)
Farmers	172	64 (37.2)	50 (29.1)	55 (17.4)
Civil servants	52	5 (9.6)	0 (0.0)	33 (5.8)
Others	96	65 (67.7)	12 (12.5)	0 (0.0)

[1]N = total number of positive samples, [2]*C. violaceum = Chromobacterium violaceum*,[3] *E. coli = Escherichia coli*, [4]*S. aureus= Staphylococcus aureus*

Table 2: Distribution of isolate by age group ([1]N= 702)

Age group (yrs)	Number examined	Number (%) positive for isolates		
		[2]*C. violaceum*	[3]*E. coli*	[4]*S. aureus*
< 10	0	0 (0.0)	0 (0.0)	0 (0.0)
11-20	104	60 (57.7)	14 (13.5)	13 (12.5)
21-30	309	147 (47.6)	76 (24.6)	59 (19.1)
31-40	244	80 (32.8)	63 (25.8)	53 (21.7)
41-50	45	10 (22.2)	3 (6.7)	14 (31.1)
>50	0	0 (0.0)	0 (0.0)	0 (0.0)

[1]N = total number of positive samples, [2]*C. violaceum = Chromobacterium violaceum*,[3] *E. coli = Escherichia coli*, [4]*S. aureus= Staphylococcus aureus*

Table 3: The percentage susceptibility profile of 3 different strains of bacterial isolates against some commonly available antibiotics in Ekpoma

Antibiotics	Number (%) positive for		
	[1]*C. violaceum*[4]n=297	[2]*E. coli*[4]n=156	[3]*S. aureus*[4]n=139
Ciprofloxacin	240 (80.8)	96 (61.5)	45 (32.4)
Ofloxacin	243 (81.8)	103 (66.0)	55(39.7)
Sparfloxacin	230 (77.4)	110 (70.5)	100 (71.9)
Gentamicin	176 (59.3)	86 (55.1)	52 (37.4)
Ampiclox	102 (34.3)	72 (46.2)	40 (28.7)
Cotrimoxazole	136 (45.8)	68 (43.6)	32 (23.0)
Oxacillin	170 (57.2)	70 (44.9)	52 (37.4)
Ceftriaxone	38 (12.8)	86 (55.1)	87 (62.6)
Cefuroxime	40 (13.5)	100 (64.1)	84 (60.4)
Rifampicin	[5]*	[5]*	90 (64.7)
Erythromycin	[5]*	[5]*	52 (37.4)

[1]*C. violaceum = Chromobacterium violaceum,* [2]*E. coli = Escherichia coli,* [3]*S. aureus= Staphylococcus aureus,*[4] *n* = total number of positive samples, [5]* = antibiotic was not used against this isolate

Table 4: Distribution of Isolates from 702 samples of females with recurrent vaginal discharge in Ekpoma (N[1] =702)

Isolates	Number (%) positive for isolates
Chromobacterium violaceum	176 (25.1)
Staphylococcus aureus	62 (8.8)
Escherichia coli	81 (11.5)
Chromobacterium violaceum and Staphylococcus aureus	46 (6.6)
Chromobacterium violaceum, Staphylococcus aureusand Escherichia coli	31 (4.4)
Escherichia coli and Chromobacterium violaceum	44 (6.3)
Candida albicans	112 (16.0)
Trichomonas vaginalis	31 (4.4)
Chromobacterium violaceum, Staphylococcus aureus, Escherichia coli and Candida albicans	0 (0.0)

[1]*N= Total number of samples examined.*

Discussion

Between the age of puberty and menopause, various risk factors may predispose females to vaginal discomfort and discharge. In Ekpoma and many parts of Africa, vaginal discharge is normally not seen as a serious disease condition because it most often stops on slight application of antimicrobial agents. It then becomes a cause for concern when a recurrent episode is experienced after several attempts are made to treat it without success. This investigation (Table 4) reveals 42.4% incidence of a rare *Chromobacterium violaceum* involvement in recurrent vaginal discharge (occurring both as a sole agent of infection and in association with other microbes). Ever since the first human infection was reported in 1927 in Malaya, there is scarcely any other report especially in sub-Saharan Africa, except 2 cases in Western Nigeria and Senegal.[3,15,16] To the best of our knowledge, this may be the first report in Nigeria of involvement of *Chromobacterium violaceum* in recurrent vaginal discharge.

This observed involvement of *Chromobacterium violaceum* in vaginal discharge may be of public health importance to health care providers in Ekpoma which has few private clinics with no specialist or referral hospital. There

is no documented report of *C. violaceum* involvement in vaginal discharge in this region. Many cases are taken to be due to candidiasis or vaginosis by *Gardnerella vaginalis*. When attempt is made to culture the discharge, detailed identification that could have warranted in-depth sensitivity testing on isolates is not done, due to poor resources. Healthcare providers especially in Ekpoma therefore resort mostly to empirical use of cheap broad-spectrum antibacterial (Septrin) and antifungal (Nystatin) agents in attempt to assist patients with this problem. Previous study in this region by Agwu et al[17] reveals significant co-infection of bacterial agents of disease (pneumococcal infection among tuberculosis patients). Empirical management of clinically diagnosed infection without full laboratory investigation due to poor diagnostic facilities could be complicated by undetected underlying infections. This may help explain the emergence of *C. violaceum* in recurrent vaginal discharge in this region.

Underlying defects in host defenses which may result from several health complications such as diabetes mellitus, pregnancy, pelvic inflammatory disease, sickle cell disease and even HIV early infection seem to predispose to infection.[18-21]

C. violaceum is a soil and water inhabitant, and it is abundant in tropical and subtropical freshwater. It is especially prevalent in water that is stagnant or slow-moving.[3,5] Ekpoma is known to have a general problem of water shortage. About 75% of the inhabitants depend on underground water reservoirs stored during rainy season and also on water supplied by water tanker drivers from nearby streams. There is no treated pipe borne water in Ekpoma.

The source of infection of *C. violaceum* among females in Ekpoma was not very clear. There was no evidence of fresh wound infection or septicaemia among the study population which would have given a clue on infection source. We hypothesize soil and water contamination of the genital tract during compulsory monthly environmental sanitations done mostly by females and also recreational activities at few stagnant streams available in the community. In relation to previous reports[22] that relapse of the disease may be due to the presence of internal organ abscesses, introduction of the bacterium to small abrasions in the genital tract during sex could also be a possibility especially in this era of prevalent low immunity in sub-Saharan Africa mostly due to HIV and malnutrition. We also suggest contamination of vaginal tract during insertion of intra uterine devices, contraceptive pills and use of poor quality contaminated condoms during sexual intercourse. The presence of *E. coli* and *S. aureus* may depict feacal contamination of genital tract due to poor hygiene and the act of cleaning from anus to the vulva after defecation.[23] The observed *C. albicans* may be due either to resistance to antimicrobial agents or is just part of vaginal normal flora.

The incidence of *C. violaceum* was highest (tables1 and 2) among traders, students and farmers with 51.5%, 46.8% and 37.2% respectively. This corresponds to the high incidence observed in age groups 11-20 and 21-30 years with 57.7% and 47.6%, since traders and students in this region mostly fall within the age group 11-30 years.

There was no significant difference in prevalence of infection using the Randomized Block Design (RBD) at (α = 0.1) when prevalence of bacterial isolates were compared among different occupational groups despite the different percentage prevalence observed in different occupational settings. This means that occupation has no relevance in prevalence of isolates among subjects. There was also no significant difference in prevalence when distribution of bacterial isolates was compared among themselves. This implies that the rate of occurrence of one isolate

within the same habitat per unit time is independent of the presence of other isolates.

On the other hand, there was a significant difference using the Randomized Block Design (RBD) at ($\alpha = 0.1$) when rate of occurrence of bacterial isolates were compared among age groups. This implies that different age groups vary in the way they respond to infection by *C. violaceum* and other bacterial isolates in this investigation. This is expected as economic, social and immunological factors enhancing disease spread may vary with age. One instance of possible reason for the outbreak of *C. violaceum* among age group 11-30 years may be ascribed to high sexual active life, coupled with marital and childbearing age, which make lots of normal flora and pathogenic bacteria to thrive well in their genital tract[2]

Our observation is different from 49% *Gardnerella varginalis* and 28% Gonococcus reported by Nnaji and his colleagues in Abakaliki, Ebonyi State.[7] The absence of *Gardnerella varginalis* and Gonococcus may be due to better preventive and control strategy due to more awareness of their mode of spread. Another possibility could be their susceptibility to broad-spectrum antibiotics often used in an attempt to treat the discharge presumptively without proper diagnosis or due to social and geographical difference between Ekpoma and Abakaliki where these two investigations were conducted.

C. violaceum generally showed the highest sensitivity to the flouro-quinolones with ofloxacin (81.8%) toping the list and ciprofloxacin following with (80. 8 %) (Table3). This is in line with previous report from Honkong.[24] *C. violaceum* was poorly susceptible to cephalosporin. *E coli* also showed the highest susceptibility to the flouro-quinolones used in this investigation with sparfloxacin 110 (70.5%) being on top. *S. aureus* which showed 100 (71.9%) sensitivity to sparfloxacin was the highest recorded among the antibiotics used for *S. aureus*.

Though the optimal antibiotic regimen is not well known[6] some studies advocate the use of parenteral antibiotics for an extended period, followed by at least 4 weeks with an oral agent, such as tetracycline, to prevent relapse[6] *C violaceum* is usually susceptible to *in vitro* chloramphenicol, tetracycline and gentamicin. It is variably sensitive to penicillins and aminoglycosides but is resistant to most cephalosporins. Erythromycin seems to be ineffective in vivo regardless of susceptibility testing.[24] The observed high efficacy of fluoro-quinolones in general, against *C. violaceum* is different from previous reported resistance in Canada.[25] and also is promising in effective management and control of the emerging outbreak. This shows that the microbial (*Streptococcus pneumoniae*) resistance to quinolone observed by Agwu et al [26] in Ekpoma does not apply to *C. violaceum* yet. Multiple drug therapy and antibiotic surveillance should be initiated quickly in Ekpoma to help prevent development of drug resistance by *C. violaceum* as previously highlighted[27]

Oxacillin and ampiclox showed sensitivity of 57.2% and 34.3 % respectively to *C. violaceum*. The mechanism of penicillin resistance to isolate is not clear. It may not be a beta-lactamase mechanism since some normal vaginal flora may prevent *C violaceum* eradication by destroying penicillin, but may be based on antibiotic tolerance where the growth of tolerant bacteria is inhibited by relatively low concentration of antibiotic, but extremely higher concentrations are needed to kill the organism.[28] Again, elaborate surveillance studies and a coordinated multidisciplinary approach is required to halt the emergence of penicillin resistant *C. violaceum* in this region.

From Table 3, the observed 59.3% sensitivity to gentamicin is in line with the reported susceptibility of isolate to gentamicin.[25] The difference in susceptibility of isolates to quinolone (ciprofloxacin) 80.8% on one hand and aminiglycoside (gentamicin) 59.3% on the other hand may be explained by the availability and high rate of abuse of gentamicin due to low cost and availability.

There was a significant difference in the sensitivity of isolates to different antibiotics when they were compared by using Completely Randomized Design[14] for unequal number of observations at ($\alpha = 0.1$). This again is expected as different antibiotics have different susceptibility patterns (Table 3).

In conclusion, human infections caused by *C. violaceum* are rare but when they occur, are usually fatal. Surveillance with improved diagnostic facilities can increase awareness among health care providers on this form of infection. A high index of suspicion is required for diagnosis, especially in the presence of a history of outdoor activities. Appropriate systemic antimicrobial therapy to halt progression of infection is mandatory, even when the infection appears to be localized in the genital tract.

References

1. Healthlink worldwide. All About STDs and AIDS. Action Newsletter, 1994; Issue 16.
2. Wooley PG. *Bacillus violaceum manilae* (a pathogenic organism). *Bull Johns Hopkins Hosp.* 1905; 16: 89.
3. Sneath PHA, Whelan JPF, Singh RB, Edwards D. Fatal infection by *Chromobacterium violaceum. Lancet*. 1953; 2: 276–7.
4. Macher AM, Casale TB, Fauci AS. Chronic granulomatous disease of Childhood and *Chromobacterium violaceum* infections in the South-Eastern United States. *Ann Intern Med.*1982; 97: 51-5.
5. Bradley D, Bilton MD, Lester W, Johnson MD. Louisiana State University. Recurrent Nonfatal *Chromobacterium violaceum* Infection in a Nonimmunocompromised Patient. *Infect Med*. 2000; 17(10): 686-689.
6. Martin WJ, Martin SA. Carlimatobacterium, Cardiobacterium, Chromobacterium and streptobacillus, In Collier, L., Ballows, A., Sussman, M., Topley and Wilson's Microbiology and Microbial Infections. Systematic Bacteriology, Volume 2, Arnold Publishers, London. 1998; 1217-1229
7. Nnaji J, Afoke A, Moses ALO. Association of Bacterial Vaginosis to Human Immunodeficiency Virus (HIV) Infection among Women in Abakaliki Metropolis. *Journal of Biomedical Science in Africa.* 2004; 2(1): 35-37.
8. Barrow GI, Feltham RKA. Cowan and Steel's Manual for the Identification of Medical Bacteria. 1993, 3rd ed. Cambridge University Press, UK.
9. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2004; Vol II, 2nd ed. Cambridge University Press, UK.
10. Raphael SS. Lynch's Medical Laboratory Technology. 1983; 4th Ed. WB Saunders Company London.
11. National Committee for Clinical Laboratory Standards, Performance Standards for antimicrobial disc susceptibility tests. Wayne, Pennsylvania National Committee for Clinical Laboratory Standards. NCCLS document M2-A7, 2000.
12. National Committee for Clinical Laboratory Standards. Performance Standards for antimicrobial disk susceptibility tests: approved standard M2-A6. 1997; Wayne, PA: National Committee for Clinical Laboratory Standards.
13. National Committee for Clinical Laboratory Standards. Performance Standard for antimicrobial susceptibility testing: eleventh in-

formational supplement. Wayne Pennsylvania National Committee for Clinical Laboratory Standards. NCCLS Documents M100 –S11, 2000.

14. Randomized block designs. 5.3.3.2. *NIST/SEMATECH e-Handbook of Statistical Methods*. 2006. Available at http://www.itl.nist.gov/div898/handbook/.
15. Onile A, Sobowale BO, Odugbemi T. Human infection due to *Chromobacterium violaceum*: a report from Ilorin, Nigeria. *East Afr Med J*. 1984; 61: 849-52.
16. Dromigny JA, Fall AL, Diouf S, Perrier-Gross-Claude JD. *Chromobacterium violaceum*: a case of diarrhea in Senegal. *The Pediatric Infectious Disease Journal.* 2002; 21(6): 573-574.
17. Agwu E, Ohihion AA, Agba MI, Okogun GRA, Okodua M, Tatfeng YM, Nwobu GO. Incidence of Streptococcus Pneumoniae Infections Among Patients Attending Tuberculosis Clinics in Ekpoma, Nigeria. *Shiraz Electronic Medical Journal (SEMJ)*. 2006;7(1):1-8.
18. Alexander HG, Paolo R, Kim J. Analysis of HIV and CMV specific memory T-cell response during primary and chronic infections. *Blood*. 2002;100(4):1381-1387.
19. Angel JB, Parato KG, Kunner A. Progressive HIV specific immune recovery after prolonged viral suppression. *Journal of infectious Disease*. 2001;183(4):546-554.
20. Balint GA. Situation analysis of HIV/AIDS epidemic in sub-Saharan Africa. *East African Medical Journal.* 1998;75(12):684-696
21. Newton ER, Piper J, Pears W. Bacteria vaginosis and intra amniotic infections. Up date. AMF Physician. *AMJ Obster Gynecol.* 1997;176:672-675.
22. Sirinavin S, Techasaensiri C, Benjaponpitak S, Pornkul R, Vorachit M. Invasive *Chromobacterium violaceum* infection in children: case report and review. *Pediatr Infect Dis J.* 2005;24:559-61.
23. Agwu E, Agba MI, Nwobu GO, Isibor JO, Okpala HI, Ohihion AA, Uzoaru SC. Pattern of Bacterial Urinary Tract Infection Among Female Students of National Veterinary Research Institute Vom. *Journal of Biomedical Science in Africa*. 2004;2(2):15-17.
24. Teoh AYB, Hui M, Ngo KY, Wong J, Lee, KF, Lai PBS. Fatal septicaemia from *Chromobacterium violaceum*: case reports and review of the literature. *Hong Kong Med J.* 2006;12(3):228-231.
25. Public Health Agency of Canada. Guidelines on susceptibility testing of antibiotic-resistant *Enterobacteriaceae* due to extended spectrum beta-lactamases (ESBLs). 2002 updated.
26. Agwu E, Agba, MI, Nwobu, GO, Ongey JY, Inyang NJ, Imharebhor JA. Antimicrobial Resistance Profile of *Streptococcus pneumoniae* isolated from Suspected Tuberculosis Patients in Ekpoma and environs. *Shiraz Electronic Medical Journal (SEMJ).* 2004;5(3):1-11
27. Dutta S, Dutta SK. Multidrug resistant *Chromobacterium violaceum*: an unusual bacterium causing long standing wound abscess. *Indian J Med Microbiol*. 2003;21:217-8.
28. Nuoti JP, Butler JC, Crutcher JM, Guevara R, Welch D, Holder I. An outbreak of multidrug resistant pneumococcal pneumonia and bacteremia among unvaccinated nursing home resident. *N Engl J Med.* 1998;338:1861–8.