Bioresearch Communications
Volume 02, issue 02, July 2016.

Journal Homepage: www.bioresearchcommunications.com

Original Article

Drug Resistance, Serological Study and Plasmid Profile Analysis of Bacterial Isolates from Anorectal Sepsis

Sheikh Shahidul Islam[1], Md. Abdul Malek[1], Kaisar Ali Talukder[2], Muhammad Asaduzzaman[1], Farhana Akther[1] and Marufa Zerin Akhter[1]

[1]Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh; [2]Laboratory of Enteric Microbiology, Laboratory Science Division, International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Mohakhali, Dhaka 1212, Bangladesh.

ABSTRACT: Anorectal sepsis is a clinical state accompanied by the infection of the anorectal region of the human body and inappropriately treated sepsis may result in increased morbidity and mortality. Common anorectal sepsis cases found in this study were the anorectal abscess, anal fistula, surgical wound infection and fissure in ano. A total of 100 bacterial positive anorectal sepsis cases collected from patients of three large referral hospitals in Dhaka, Bangladesh were studied during the period from May 2006 to December 2007. Of these anorectal specimens, abscess cases were 42%, wound infection 28%, fistulae in ano were 26% and fissure in ano 4%. The microorganisms obtained from the specimens were identified by phenotypical and biochemical tests. *Escherichia coli* was the most prevalent isolate (61%) followed by *Staphylococcus aureus* (22%), *Proteus* spp. (10%) *and Pseudomonas* spp. (7%). All the *E. coli* strains isolated were totally resistant to multiple drugs including ampicillin, cotrimoxazole and nalidixic acid. However, 81%, 58% and 36% of the *E. coli* isolates were sensitive to gentamycin, ceftazidime and ceftriaxone respectively. *S. aureus* obtained from all types of anorectal sepsis were sensitive to gentamycin (79%), ceftazidime (46%), ceftriaxone (28%), ciprofloxacin (39%), erythromycin (40%), penicillin (19%), tetracycline (14%) and cephalexin (25%). *S. aureus* showed 100% resistance to cloxacillin. All the *Proteus* isolates were totally resistant to penicillin, amoxicillin and cotrimoxazole. However, 55%, 44%, 38% and 25% of these *Proteus* isolates were sensitive to ceftazidime, gentamicin, ceftriaxone and ciprofloxacin respectively. The isolated *Pseudomonas* spp. showed 67%, 63%, 45% and 25% sensitivity to gentamycin, ceftriaxone, ceftazidime, ciprofloxacin respectively and absolute resistance to penicillin, amoxycillin, cotrimoxazole, cephalexin, cephradine, ampicillin, nalidixic acid and nitrofurantoin. In this study, the most prevalent serotype of *E. coli* was found to be O25 and O20 and the other isolates of *E .coli* were untypable. In plasmid profile analysis of 14 randomly selected *E. coli* isolates, 10 different plasmid patterns ranging from 1 to 140 MDa were observed. However, no correlation could be ascertained between plasmid pattern and drug resistance.

KEYWORDS: multi drug resistance, anorectal sepsis, serotype, plasmid

CITATION: Islam, S. S., Malek, M. A., Talukder, K. A., Asaduzzaman, M., Akther, F., Akther, M. Z., 2016. Drug Resistance, Serological Study and Plasmid Profile Analysis of Bacterial Isolates from Anorectal Sepsis. *Biores Comm.* **2**(2), 270 - 275.

CORESPONDENCE: Dr. Marufa Zerin Akhter, E-mail: mzakhter@du.ac.bd

INTRODUCTION

Anorectal sepsis is a common surgical emergency. Severe bacterial infection of the anorectal region causes anorectal sepsis. The anorectal region of the human body is the lower portion of the large intestine between the sigmoid colon and anal canal. Bacterial infection in this region is called anorectal sepsis which is associated with disease conditions involving perianal abscess, fistula, fissure in ano, hemorrhoid, proctitis, ischiorectal abscess and surgical wound infection[1].

Bacterial pathogens that have been isolated from anorectal sepsis are *E. coli*, Enterobacteria, *Staphylococcus aureus*, *Proteus*, *Pseudomonas*, *Salmonella* spp. and gut-specific anaerobes[2,3]. *E. coli* is

the most common member of the family Enterobacteriaceae that accounts for the majority of the perianal abscesses and wound infectious of anorectal sepsis[4, 5].

Sepsis may take place in cases of immunosuppressive, diabetes, Crohn disease and ulcerative colitis. Timely and appropriate treatment prevents more serious complications as an extension of the anorectal sepsis or abscess or serious systemic infection[6,7,8]. Antibiotic resistance is one of the major causes of failure in the treatment of infectious disease that results in increased morbidity, mortality and cost of health care[9].

Bangladesh is a densely populated developing country and most of the people suffer from ignorance, illiteracy and malnutrition[10]. Poor knowledge of anal hygiene, inadequate number of toilets, life style, poor economic status, food habit, lack of safe disposal of excreta etc. predispose disease condition of the anorectal region and the bacteria take the opportunity in infecting the anorectal region. In addition, lack of proper education and training of the doctors and mal practice of the concerned practitioners also play a role in the occurrence of frequent infection[11].

The continuing emergence of pathogenic microorganisms that are multidrug resistance (MDR) is a cause of increasing concern. Systematic study is necessary to determine the prevalence of MDR *E. coli* and other organisms because our recent studies revealed the strong correlation between MDR *E. coli* and extended spectrum beta lactamase (ESBL) producing *E. coli* in anorectal sepsis cases[12]. The present study was conducted to determine the pattern of microbial flora present in the samples from anorectal sepsis patients. After initial identification by culture, microscopy, and biochemical tests, the organisms were subjected to antibiogram and serotyping. Being the most prevalent isolate of multi drug resistant bacteria, *E. coli* implicated the necessity to study the overall characteristics of this bacterium by using both phenotypic and genotypic techniques. Plasmid profile analyses of some selected strains of *E. coli* were performed. According to the modified Kauffman [13] scheme, *E. coli* are serotyped on the basis of their O (somatic), H (flagellar) and K (capsular) surface antigen profiles[14]. *E. coli* of specific serogroups can be associated reproducibly with certain clinical syndromes, but it is not in general the serologic antigens themselves that confer virulence. Rather, the serotypes and serogroups serve as readily identifiable chromosomal markers that correlate with specific virulent clones[15].

The present study also covered the plasmid profile analysis to correlate the presence of plasmids with virulence which would allow to comprehend the molecular mechanism of the isolates by which resistance gene are acquired or transmitted that might contribute to the creation of new antimicrobial strategies as well as to acquire newer preventive measures to stop further spreading of resistance determinants among the pathogens[16].

MATERIALS AND METHODS

Collection of Samples

The study was conducted from May 2006 to December 2007 and the samples were collected from Dhaka Medical College Hospital, Bangabandhu Sheikh Mujib Medical University and Japan Bangladesh Friendship Hospital located in Dhaka city. A total of 125 samples comprising pus, exudate, and rectal swabs were collected from patients of anal abscess, fistula, post-surgical wound (after haemorrhoidectomy, incision and drainage of different origin) and anal fissure following aseptic technique and transferred to the laboratory using special transport medium (thioglycollate broth medium) at the earliest convenience.

Bacterial isolation

Various commercial media were used for the isolation and characterization of bacterial isolates e.g. blood agar, MacConkey agar, mannitol salt agar, nutrient agar, cetrimide Agar, eosin methylene blue agar and Loeffler's serum[17,18,19]. The pure culture was transferred to appropriate media mixed with sterile 80% glycerin and used as stock culture that were preserved at -20°C.

Microscopic examination

Microscopic examinations of the isolates were performed for the determination of bacterial size, shape, arrangement, presence of endospore, capsule and staining properties[20].

Identification of bacteria by conventional biochemical tests

Isolated bacteria were identified by standard laboratory biochemical tests according to the methods described elsewhere[21]. The biochemical tests for *E. coli* were brilliant green lactose bile broth test, indole test, citrate utilization test, methyl red test, Voges-Proskaur test, Kilgler's iron agar test and nitrate reduction test. Biochemical characteristics of *S. aureus* were determined by motility, sugar fermentation, urea, catalase, nitrate and methyl red test. For analysis of the biochemical characteristics of *Proteus*, gram staining, motility, lactose, urea and phenylalanine agar tests were carried out. In case of *Pseudomonas*, gram staining, motility, sugar fermentation tests were performed.

Serotyping

All the *E. coli* isolates (n=24) were serologically confirmed by using commercially available antisera kit (Denka Saikn, Co. Ltd., Japan). Isolates were subcultured on Tripticase soy agar (Difco, Becton-Dickinson & Sparks, USA) plates and after about 18h of incubation; the serological reaction was performed by the glass slide agglutination test as described by Sakazaki[22].

Antibiotic Susceptibility test

Bacterial susceptibility to antimicrobial agents was done in vitro by employing the standardized agar-disc-diffusion method[23]. In this process bacteria were categorized resistant or susceptible to each antimicrobial agent following the standard chart[24]. Antibiotics (Oxoid Ltd., England) and the disc potency used were: Penicillin (10U) ampicillin (10 µg), amoxycillin (25 µg), tetracycline (30 µg), erythromycin (15 µg), cloxacillin (30 µg), gentamycin (10 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), cephalexin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cotrimoazole (25 µg), cephradine (30 µg), nitrofurantoin (300 µg).

Plasmid DNA profiling

Plasmid DNA was isolated following the alkaline lysis method of Kado and Liu[25]. Plasmid DNA preparations were electrophoresed through a horizontal gel apparatus from MAX Submarine Agarose gel unit, HE 99 (California, USA) in a 0.7% agarose gel (50 V at room temperature for about 2 hr). The gel containing the plasmid DNA was first stained with ethidium bromide and then visualized in Gel-Doc.

RESULTS AND DISCUSSION

Anorectal sepsis has long existed as a problem that results from the impediment of anal glands with subsequent retrograde infection[7]. In Bangladesh, morbidity and mortality due to anorectal sepsis leading to malignancy are an increasing burden to the society. However, due to the lack of microbiological research at the molecular level on the etiology and changing patterns of antibiogram of anorectal pathogens, the appropriate intervention, treatment and preventive measures have become an increasing problem for the clinicians[11,12]. This study had been designed to investigate the drug resistance pattern and the frequency of plasmids as well as the relationship between antibiotic resistance and plasmids carriage of the MDR *E. coli* isolates in anorectal sepsis patients. Other aetiological bacteria and their antibiograms were also investigated. In the present study, a total of 125 samples from anorectal sepsis of various types were examined for identification of the organisms of which 100 (80%) were positive as to contain bacterial isolates. Out of the one hundred samples, anorectal sepsis, anorectal abscess, anal fistula, wound infection, and fissure in ano cases were 42%, 26%, 28% and 4% respectively (Table 1). The common etiological agents of anorectal sepsis are *Bacteroides, Pseudomonas, E. coli, Proteus, Streptococcus β hemolytic, Staphylococcus aureus* and rarely tubercle bacilli and gonococci[26]. A recent study showed that *E. coli*, *Enterococcus a*nd *Klebsiella pneumoniae* were the leading pathogens[5]. However, in contrast to these findings, the present study found *E. coli* as the most predominant bacterial isolate which was (61%) and the second leading pathogen as *S. aureus* (22%) followed by *Proteus* (10%). *Pseudomonas* comprised only 7% in this study (Table 1). In two previous studies carried out by Vanhueverzwyn *et al.*[27] and Barnes and colleagues[28], *Pseudomonas* was reported to be the most common organism isolated from samples of abscess fluid or blood. The present study demonstrated that aerobic bacteria are the most frequently isolated organisms in these infections which were similar to some previous findings[29, 30].

Table 1. Prevalence of bacteria according to the type of anorectal sepsis cases (Total no. of positive cases=100).

Bacterial isolates	Anorectal sepsis cases				
	Anorectal abscess	Anal fistula	Surgical wound (Infected)	Fissure in ano	Total (percentage)
E. coli	22	17	20	2	61 (61%)
S. aureus	12	3	5	2	22 (22%)
Proteus	2	5	3	None	10 (10%)
Pseudomonas	6	1	None	None	7 (7%)
Total (percentage)	42 (42%)	26 (26%)	28 (28%)	4 (4%)	

The use of antibiotics for the treatment of anorectal sepsis ideally requires the isolation of the bacterium and a determination of its antibiotic sensitivity. There are two approaches to antibiotic treatment. A narrow spectrum antibiotic may be used to treat a known sensitive infection. Combinations of broad spectrum antibiotics can be used when the organism is not known or when it is suspected that more than one bacterium may be responsible for infection acting in synergy. In the present study, all the organisms isolated from anorectal sepsis were tested for antibiotic sensitivity. Figure 1 (a-d) shows the variable patterns of antibiotic susceptibility of the isolated bacteria. In the present study, about 20% *Escherichia coli* were found to be resistant to gentamycin which is similar to a previous study carried out by Steigbrigel *et al.*[31] which reported about 10-35% of *E. coli* being resistant to aminoglycosides. In *Staphylococcus aureus* sensitivity pattern, Osoba and coworkers[32] reported that *Staphylococcus aureus* was mostly resistant to ampicillin and amoxycillin, but highly sensitive to ceftriaxone; however, in the present study, the sensitivity to ceftriaxone was found to be only 28%. The susceptibility pattern of *Proteus* found in this study was similar to Osoba and coworkers[32] who reported that *Proteus* strains were highly susceptible to ceftriaxone but resistant to ampicillin and amoxycillin. The antibiogram findings of *Pseudomonas* were similar to the works of William *et al.*[33] who reported that the organism were moderately susceptible to gentamycin (50%), but highly resistant to ampicillin. However, tetracycline resistance were moderate (50%) according to these researchers; whereas, in the present study, it was 100%.

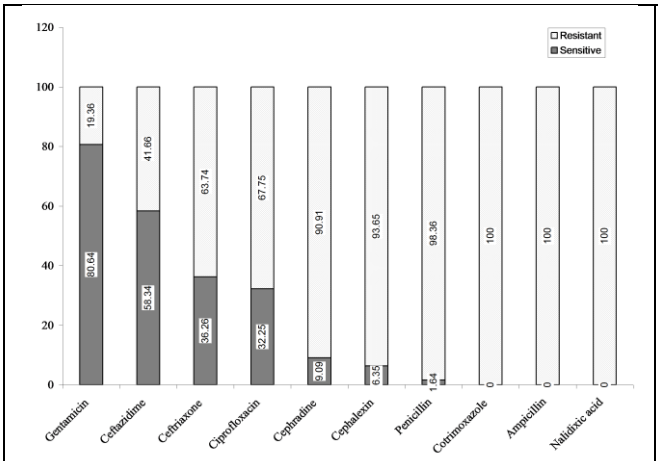

Figure 1a. Antibiotic susceptibility pattern of *E. coli* isolates.

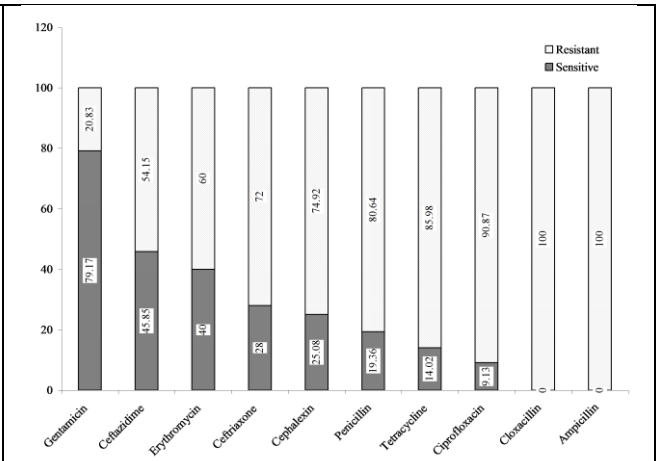

Figure 1b. Antibiotic susceptibility pattern of *S. aureus* isolates.

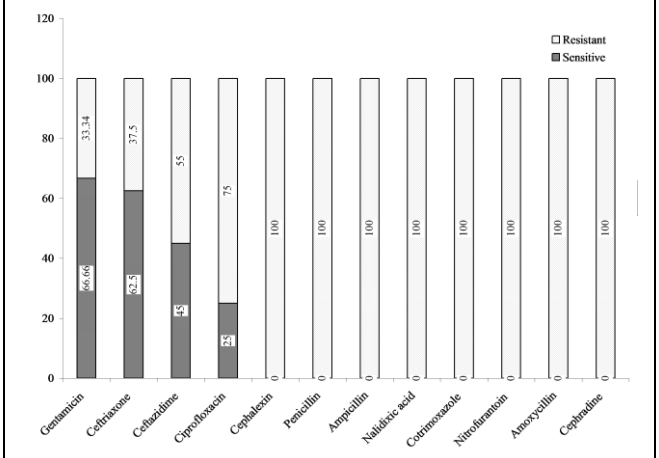

Figure 1c. Antibiotic susceptibility pattern of *Proteus* spp. isolates.

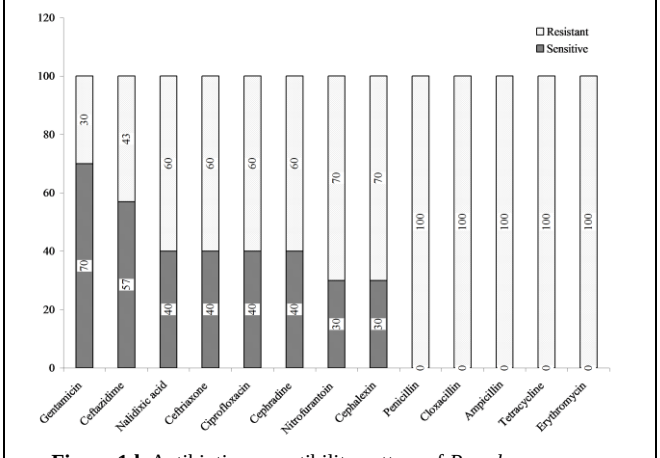

Figure 1d. Antibiotic susceptibility pattern of *Pseudomonas* spp. isolates.

The pattern of antibiogram of the present study showed gentamycin to be the most potent drug in case of the treatment of anorectal sepsis. The second most potent drug was identified as ceftazidime. Ceftriaxone was found to be the third potent drug which is almost similar to the result of Osoba *et al.*[32]. All the organisms isolated from anorectal sepsis were found to be multidrug resistant. Most of the isolates were resistant to at least three drugs and maximum isolates were resistant to 6 or 7 drugs whereas some of them were found to be resistant to all drugs. *E. coli*, *S. aureus*, *Proteus* and *Pseudomonas*, all the organism isolated from anorectal sepsis showed multidrug resistance and many of the isolates of *E. coli* have been identified as all drug resistant including resistant to 3rd generation cephalosporins.

The *E. coli* isolates of this study were identified primarily by employing standard serological methods using commercially available antisera (Denka Saiken Co Ltd., Japan). The serological classification scheme is based on the antigenic differences in highly variable bacterial surface molecules[34]. Because most strains of *E. coli* are not pathogens and because different strains cause different types of diseases, it is important to be able to differentiate strains or groups of strains so that strains responsible for a particular outbreak can be identified. The results of the investigation are presented in Table 2. In this study, the most prevalent serogroup was O25 followed by O20. Other isolates were different and they were designated as untypable.

Table 2. Serological characteristics of *E. coli* isolates tested with commercially available antisera (n=24).

Isolates	E 02	E 03	E 04	E 07	E 12	E 14	E 15	E 20	E 22	E 24	E 25	E 26	E 28	E 29	E 30	E 35
Poly 5		O25			O25	O25	O25	O25	O20			O20				
Untypable	√		√	√						√	√		√	√	√	√

All the multidrug-resistant (MDR) isolates of *E. coli* were examined for the presence of plasmids. Analysis of plasmid profiles is useful tools to document the appearance of the plasmid with important phenotypic characteristics, most importantly the drug resistance. Plasmids are responsible for the transfer of drug resistance to other organisms in hospital and community[35]. Analysis of plasmid DNA by agarose gel electrophoresis revealed that all the isolates contained multiple numbers of plasmid ranging from 1 to 140 MDa, forming multiple banding patterns. Some showed a band with molecular weight >140MDa. Among 14 isolates, 9 patterns of plasmid were found. Pattern 1 and pattern 7 constitute 35.71% (n=5) and 4.29% (n=2) of all plasmids respectively. On the other hand, each of the patterns 2, 3, 4, 5, 6, 8 and 9 constitutes 7.14% (n=1) of total plasmid (Figure 2 and Table 3). The plasmid patterns were diverse in *E. coli.*

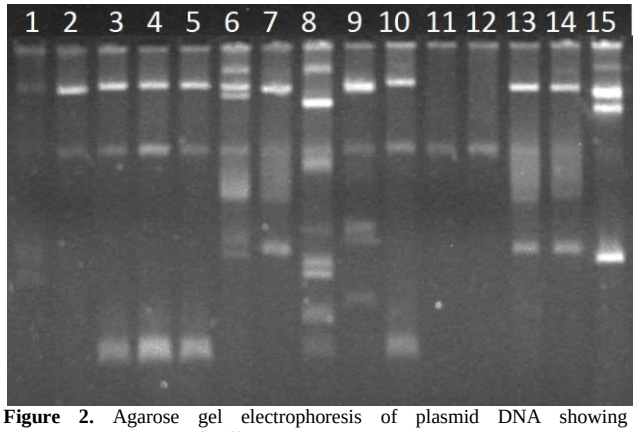

Figure 2. Agarose gel electrophoresis of plasmid DNA showing representative pattern of different *E. coli* isolates. Lanes: 1, *E. coli* PDK-9 (marker); Lanes: 2, *E. coli* 02; Lanes: 3, *E. coli* 07; Lanes: 4, *E. coli* 12; Lanes: 5, *E. coli* 14; Lanes: 6, *E. coli* 15; Lanes: 7, *E. coli* 20; Lanes: 8, *E. coli* 21; Lanes: 9, *E. coli* 24; Lanes: 10, *E. coli* 25; Lanes: 11, *E. coli* 26; Lanes: 12, *E. coli* 28; Lanes: 13, *E. coli* 29; Lanes: 15, *E. coli* 30.

Table 3. Plasmid profile analysis of *E. coli* isolates

Isolates	Molecular weight (in MDa)										Plasmid
	> 140	140	120	90-30	20-8	8-7	6-4	3.9-3	2.9-2.0	1.9-1.0	pattern
E02	-	+	-	-	-	-	+	+	+	+	P1
E07	-	+	-	+	-	-	+	+	+	+	P2
E12	-	+	-	-	-	-	+	+	+	+	P1
E14	-	-	-	-	-	-	+	+	+	+	P3
E15	+	+	-	-	-	-	+	+	+	+	P1
E20	-	+	-	-	-	-	+	+	+	+	P1
E21	+	+	-	-	-	-	-	-	-	-	P4
E24	-	+	-	-	-	-	-	-	+	-	P5
E25	-	+	-	-	-	-	+	+	+	+	P1
E26	-	+	-	+	-	-	+	+	+	-	P6
E28	-	+	-	-	-	-	-	+	+	-	P7
E29	-	+	-	-	-	-	-	+	+	-	P7
E30	+	-	-	-	-	-	-	+	+	-	P8
E35	-	+	-	-	-	-	+	+	+	-	P9

CONCLUSION

The most potent drugs against the bacterial isolates in the case of anorectal sepsis were found to be gentamycin, ceftazidime, ceftriaxone and ciprofloxacin in the descending order of potency. *E. coli* obtained from anorectal sepsis showed similar serotypic characters of diarrhoeagenic *E. coli* strains of O25 type (ETEC). It was observed that two *E. coli* isolates were resistant to all drugs except gentamycin. These 2 isolates had the similar serotype (O25). In this study, isolates harbored plasmids of different molecular weights with different patterns; as such, no definite correlation could be established between plasmid pattern and antimicrobial resistance. The present study have presented the antibiotic resistance pattern involved in the pathogenesis of anorectal sepsis in Bangladesh. This study might be of help to the clinicians to develop therapeutics and to plan preventive measures in anorectal sepsis cases.

REFERENCES

1. Abcarian H. 2011. Anorectal infection: abscess-fistula. *Clin Colon Rectal Surg.* **24(1):**14-21.
2. Eykyn SJ, Grace R.H. 1986. The relevance of microbiology in the management of anorectal sepsis. *Ann R Coll Surg Engl. **68:** 237-9.*

3. *Brook I. and Frazier E.H. 1997. The aerobic and anaerobic bacteriology of perirectal abscesses. J. Clin. Microbiol*. **35:** 2974–2976.
4. Liu C.K., Liu C.P., Leung C.H. and Sun F.J. 2011. Clinical and microbiological analysis of adult perianal abscess. *J Microbiol Immunol Infect*. **44:** 204–8.
5. Chen C.Y., Cheng A., Huang S.Y., Sheng W.H., Liu J.H., Ko B.S., Yao M., Chou W.C., Lin H.C., Chen Y.C., Tsay W., Tang J.L., Chang S.C. and Tien H.F. 2013. Clinical and microbiological characteristics of perianal infections in adult patients with acute leukemia. *PLoS One*. **8:** e60624.
6. Genua J.G. and Vivas D.A. 2007. Management of nonhealing perineal wounds. *Clin Colon Rectal Surg*. **20:** 322–328.
7. Ulug M., Gedik E., Girgin S., Celen M.K. and Ayaz C. 2010. *Braz J Infect Dis*. **14:** 225-229.
8. Pini Prato A., Castagnola E., Micalizzi C., Dufour C., Avanzini S., *et al*. 2012. Early diverting colostomy for perianal sepsis in children with acute leukemia. *J Pediatr Surg.* **47:** e23–7.
9. Bouza E. and Cercenado E. 2002. *Klebsiella* and *Enterobacter*: Antibiotic resistance and treatment implications. *Semin. Respir . Infect*. **17:** 215-230.
10. Haseen F. "Malnutrition among Bangladeshi women in ultra poor households: prevalence and determinants", M.Sc. Thesis in international health, International maternal and child health. Department of women's and children's health, Uppsala University, Uppsala, Sweden, pp.4, 2010.
11. Sarkar H., Hassan M. and Laila R.N. 2008. Pattern of Anorectal Disorders in Surgical Practice in Rajshahi. *Journal of Teachers Association*. **21:** 69-72.
12. Islam S.S., Malek M.A., Haque A.K.M.F., Talukder K.A. and Akhter M.Z. 2013. Beta lactamase genes of extended spectrum beta lactamase producing *Escherichia coli* from anorectal sepsis cases in Bangladesh. *Bangladesh J Microbiol*. **30:** 23-29.
13. Kauffmann F. 1944. The basis of O Antigen Serotyping is the standard tube agglutination 492 tests adapted to use U-bottomed microtitre trays. *Microbiologica Scandinavia.* **21:** 20–40.
14. Edwards P.R. and Ewing W.H. 1972. Identification of Enterobacteriaceae, 3rd edn, Burgess Publishing Company, Minneapolis.
15. Whittam T..S, Wolfe M.L., Wachsmuth I.K., Ørskov F., Ørskov I. and Wilson R.A. 1993. Clonal relationships among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. *Infection and Immunity*. **61:** 1619-1629.
16. Shapiro J.A. 1999. Views about evolution are evolving. *Am Soc Microbiol News*. **65**: 201-207.
17. Cheesbrough M. 1984. Medical laboratory manual for topical countries. 1st edn. Vol. 2, pp. 35: 40-57. Microbiology, English Language Book Society, London.
18. Carter G.R. 1986. Essentials of Veterinary Bacteriology and Mycology. 3rd edn. pp. 312-330.
19. Cowan S.T. 1985. Biochemical behavior of *E. coli*. *Journal of Genetic Microbiology*, **8:** 391.
20. Kreig N.R., Holt J.G., Williams and Wilkins. 1984. Bergeys Manual of Systematic Bacteriology, Vol. 1: 428, East Preston street, Baltimore, M.D. 21202, USA.
21. Collee J.G., Miles R.S. and Watt B. 1996.Tests for identification of bacteria. *In* Mackie and McCartney Practical Medical Microbiology. 14th edition, Collee JG, Fraser AG, Marmion BP, Simmons A (Eds.), pp.131-49, 166-7. Churchill Livingstone: New York,
22. Sakazaki R. 1992. Bacteriology of *Vibrio* and related organisms. *In:* Cholera. (Barua D and Greenough WB Eds.), pp. 37–55. *Plenum Medical* Book Co., New York.
23. Bauer A.W., Kirby W.M.M., Sheris J.C. and Truck M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Path.* **145:** 225-230.
24. Barry A.L. and Thornsberry C. 1985. Susceptibility tests: Diffusion test procedures. *In* Lennette EH, Balows A, Hausler Jr WJ, Shadomy HJ, eds. Manual of Clinical Microbiology. 4th ed, pp. 978–987. Am Soc Microbiol, Washington, D.C.
25. Kado C.I. and Liu S.T. 1981. Rapid procedure for detection and isolation of large and small plasmids. *J Bacteriol*. **145:**1365-73.
26. Russel R.C., Norman G., William S. and Christopher J.K. 2004. *In* Bailey and Love's Short Practice of Surgery, 24th edn, pp 1263-65. Hodder Arnold, London.
27. Vanhueverzwyn R., Delannoy A., Michaux J.L. and Dive C. 1980. Anal lesions in hematologic diseases. *Dis Colon Rectum*. **23:** 310–2.
28. Barnes S.G., Sattler F.R. and Ballard J.O. 1984. Perirectal infections in acute leukemia. Improved survival after incision and debridement. *Ann Intern Med.* **100:** 515–8.
29. Enberg R.N., Cox R.H. and Bury V.F. 1974. Perirectal abscess in children. *Am J Dis Child*. **128:** 360-1.
30. Krieger R.W. and Chusid M.J. 1979. Perirectal abscess in childhood: a review of 29 cases. *Am J Dis Child*. **133:** 411-2.
31. Steigbrigel N.H., McCall C.E., Reed C.W. and Finland M. 1967. Antibacterial action of 'broad spectrum' penicilins, cephalosporins and other antibiotics against Gram-negative bacilli isolated from blood cultures. *Ann. N.Y. Acced Sci.* **145:** 224-236. Australia.
32. Osoba A.O., Al-Rasheed A., Bartlett F., Khizzi N., Al-Saud A. and Al-Admany A.M. 1991. In-vitro susceptibilities to ampicillin. *Saudi. Med. J*. **12:** 309-313.
33. Salyers A.A. and Whitt D.D. 2005. *Escherichia coli* gastrointestinal infections. *In* Bacterial Pathogenesis: A Molecular Approach (Salyers AA and Whitt DD eds.), pp. 191-192. American Society for Microbiology, Washington DC.
34. Hernández J.R., Martínez-Martínez L., Cantón R., Coque T.M. and Pascual A. the Spanish Group for Nosocomial Infections (GEIH). 2005. Nationwide study of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum -lactamases in Spain. *Antimicrob. Agents Chemother*. **49:** 2122–2125.