ISOLATION RATE OF UNUSUAL BACTERIAL PATHOGENS FROM STOOL CULTURES

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SUMMARY

This study has been performed in order to determine the incidence of Aeromonas, Clostridium difficile, Yersinia enterocolitica and VETEC (Verotoxin producing Escherichia coli), in addition to routinely identified pathogens in stool cultures and to discuss necessity and cost effectiveness of the isolation procedures for these uncommon pathogens. Out of 452 stool samples collected from patients admitted to Marmara University Hospital and Haydarpaşa Numune Hospital, the isolation rates were 3% for Shigella, 1.7% for Salmonella, 0.2% for VETEC and, Yersinia enterocolitica and Aeromonas were isolated from none of the stool cultures. However C. difficile was an important pathogen in hospitalised patients with isolation rate of 12.9%.

Key Words: Diarrhea, Aeromonas, Clostridium difficile, Yersinia enterocolitica, VETEC.

INTRODUCTION

Gastrointestinal infections, particulary infectious diarrhea is one of the most important cause of mortality and morbitidy in the world. Though proper oral rehidration therapy decreases the rate of mortality, prolonged diarrhea is still the most important cause of death in developing countries (1). Severity and duration of the diarrhea, age of the patient and available laboratory fascilities contribute the clinical diagnosis therefore careful history taking and physical examination and microscopical examination of stool for the presence of fecal leukocytes are very important (2).

Many microorganisms including bacteria, viruses and protozoa may cause diarrhea. In 50-60% of sporadic cases an enteric pathogen could be recognized with the aid of developing laporatory fascilities (3).Acute diarrheal illnesses vary from mild to severe and generally clinical findings are not very helpful in suggesting etiological agent. The clinical picture is dependent upon the pathogenesis and pathopysiology of the infection. Watery diarrhea, not containing blood or leukocytes without fever suggests small bowel infection with a noninvasive enteric pathogen. In contrast, frequent diarrheal stools of small quantities which contain pus cells or blood suggest large bowel infection with an invasive microorganism.

Since the etiological agent of infectious diarrhea may be suggested by several factors, input from clinician is necessary for optimal laboratory evaluation. The laboratory cannot blindly search for all possible pathogens in the stool. The task of the clinical microbiology laboratory in the diagnosis of diarrhea is to isolate and identify those bacteria most likely to be implicated as agents of diarrhea among the fecal microflora. Table I shows the possible agents and pathologic mechanisms of diarrhea (4).

The isolation rates and spectrum of gastrointestinal pathogens are in accordance with the socioeconomical states of countries. For instance, Salmonella and Shigella spp. are the main pathogens of developing countries whereas Campylobacter spp. and other unusual pathogens are more likely to be isolated in developing countries. Among these Yersinia enterocolitica most commonly causes acute gastroenteritis with abdominal pain and diarrhea with or without blood. Other forms of Yersinia infections include: (i) Pseudoappendicitis syndrome, mesenteric lymphadenitis, or terminal ileitis, (ii) septicemia, (iii) meningitis, (iv) urinary tract infection (5). Sequelae include arthritis, erythema nodosum and, Reiter syndrome. Asymptomatic cases do occur.

Table I: Possible agents and pathologic mechanisms of diarrhea

Mechanism	Non Inflammatory (Enterotoxin)	Inflammatory (Invasion, cytotoxin)	Penetration
Localization	Proximal small bowel	Colon	Distal small bowel
Disease	Watery diarrhea	Dysentery	Enteric fever
Microscopy	Leukocytes (–)	Leukocytes (+)	Leukocytes (+)
Examples	Vibrio cholerae E. coli (LT) C. perfringens Bacillus cereus S. aureus Giardia lamblia	Shigella Invasive E coli C. difficile Campylobacter E. histolytica	S. typhi Y. enterocolitica

The pathogenic potential of Aeromonas species has been increasingly recognized over the past decade although they were first recognized as human pathogens in immunocompromised patients in 1968. The most common source for human isolates of these organisms is the gastrointestinal tract where Aeromonas species may cause gastroenteritis and where they have been recovered from bile and from hepatic abscess. They have also been recovered from wounds and soft tissue abcesses, osteomyelitis, pneumonia, peritonitis, pelvic infections (6).

Since 1982, E. coli 0157: H7 has been recognised as the most important etiologic agent of hemorrhagic colitis, which is characterized by severe abdominal pain and watery stools, followed by frankly bloody diarrhea which may be complicated with hemolytic uremic sydrome and thrombotic thrombocytopenic purpura. Hemorrhagic colitis associated with E. Coli 0157:H7 widely occur as sporadic cases, however, outbreaks related with consumption of hamburger, milky products have also been reported (7). This microorganism produces cytotoxic effects on Vero cells, hence the name VETEC has been given.

Clostridium difficile, causing clinical syndromes that ranges from asymptomatic carrier state to pseudomoembranous colitis, is responsible for 15-25% of antibiotic associated diarrhea and 99% of pseudomembranous colitis (8). Diarrhea associated with Clostridium difficile is primarily due to the usage of antimicrobials and mostly implicated agents include clindamycine, ampicillin and cephalosporin.

MATERIALS AND METHODS

Four hundred and fifty-two stool samples collected from Marmara University Hospital and Haydarpaşa Numune Hospital for isolation on enteric pathogens were transferred to the laboratory in Carry Blair transport medium. For routine studies stool samples were inoculated on MacConkey agar, SS agar and selenite broth and incubated overninght at 37°C. Suspected colonies for Salmonella and Shigella were identified by conventional techniques (9).

VETEC isolation and toxin assay: Stool samples were inoculated on Sorbitol MacConkey agar and after overnight incubation sorbitol nonfermenter colonies identified as E. coli were incubated in Penassay broth for one more night. Culture suspension was then centrifuged at 8000xg for 20 minutes and supernatant was filtrated through 22 µm Millipores. Stool filtrates were preserved at -70 °C until being processed for Verotoxin assay. For this purpose Vero cells monolayers were used and 100 µl culture filtrates of ATCC E. coli 35150 as (+) control; ATCC E. coli 25922 as (-) control and isolates of patients were added on separate wells. After 48-72 hours incubation at 37°C plates were examined under inverted microscope for typical cytotoxic effect (10).

YERSINIA ENTEROCOLITICA isolation: Stool samples were put into tubes containing 5 ml PBS and incubated at 4°C for 3 weeks (Cold enrichment). Samples are cultured on MacConkey agar on 7 th, 14th, 21st days of enrichment (11). Suspected colonies were screened by Enterotube (Roche diagnostics).

AEROMONAS isolation: Sheep blood agar supplemented with 5% ampicillin was used. After overnight incubation at 37°C suspected colonies were screened with oxidase test (12).

CLOSTRIDIUM DIFFICILE isolation and toxin assay. Stoll samples were inoculated on selective Cycloserine-Cefoxitine- fructose agar and incubated in anaerobic conditions at 37°C for 38 hours. Suspected colonies were identified as previously described (13). For toxin assay stool suspensions were centrifuged at 3000xg 20 minutes and supernatants after filtrated from 22 mm millipores were kept at -70°C until being processed. Fecal filtrates were diluted 1:1 both with sterile PBS and C. difficile antiserum and then were added 20 µl of each to separate wells. After overnight incubation at 37°C plates were examined under inverted microscope. Results were interpreted as positive if typical cytotoxic effect was observed in the former and was neutralised in the latter well. Standard Clostridium difficile culture filtrates were used as positive control (14).

RESULTS

Enteric pathogens isolated from stool samples have been given in Table II.

The presence of Clostridium difficile and VETEC was confirmed by means of their cythopathic effects on cell cultures. Cythopathic effects was characterized with cell rounding and aggregation after 24 hours incubation for Clostridium difficile. The toxic effect of Verotoxin was characterized by the rounding up of cells initially and subsequent destruction of cell monolayers with cell detachment after 48-72 hours of incubation. Results are shown in Figures 1-6.

Table II: Incidence of enteric pathogens in stool samples

	Total number / % (n:452)	Outpatients / % (n:390)	Inpatients / % (n:62)
Salmonella sp.	8/1.7	6/1.7	2/3.2
Shigella sp.	14/3	14/3.5	0
Aeromonas sp.	0	0	0
Y. enterocolitica	0	0	0
C. difficile	8/1.7	Nt*	8/12.9
VETEC	1/0.2	0	1/1.6

* Not tested

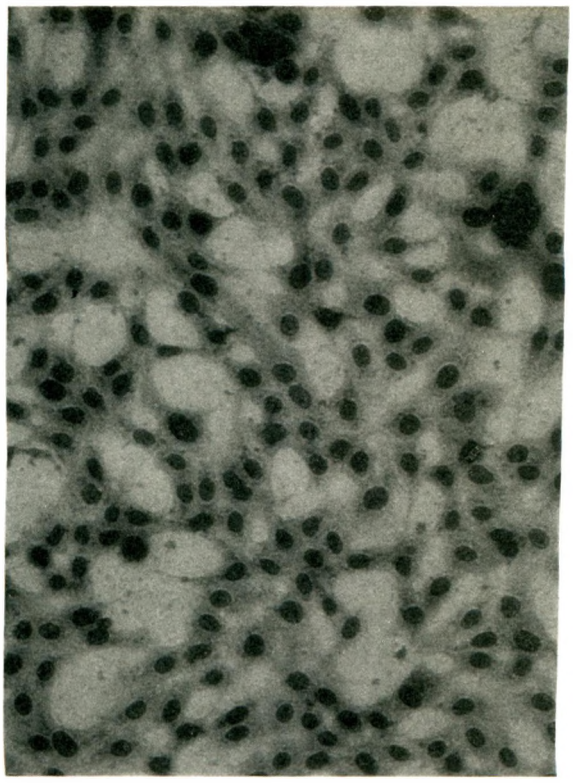


Fig.1. ATCC 35150 \ VETEC positive control \ 10 X

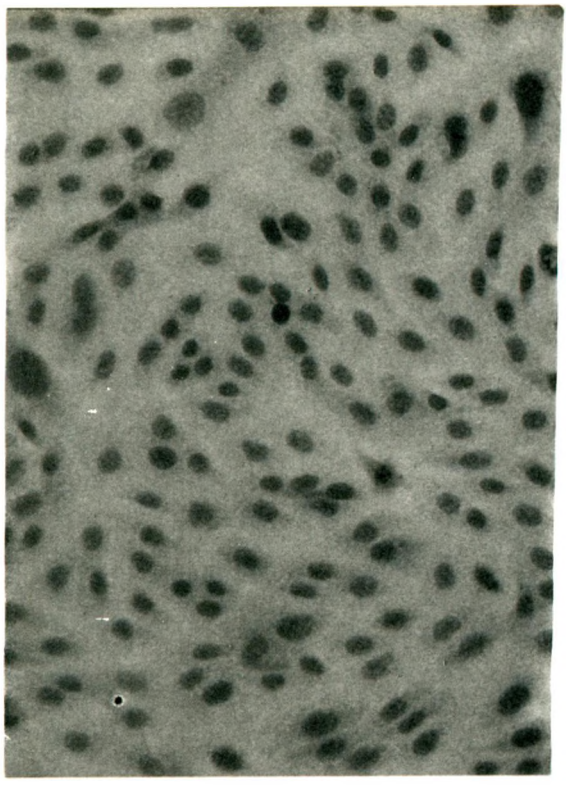


Fig.2. ATCC 25922 \ VETEC negative control \ 10 X

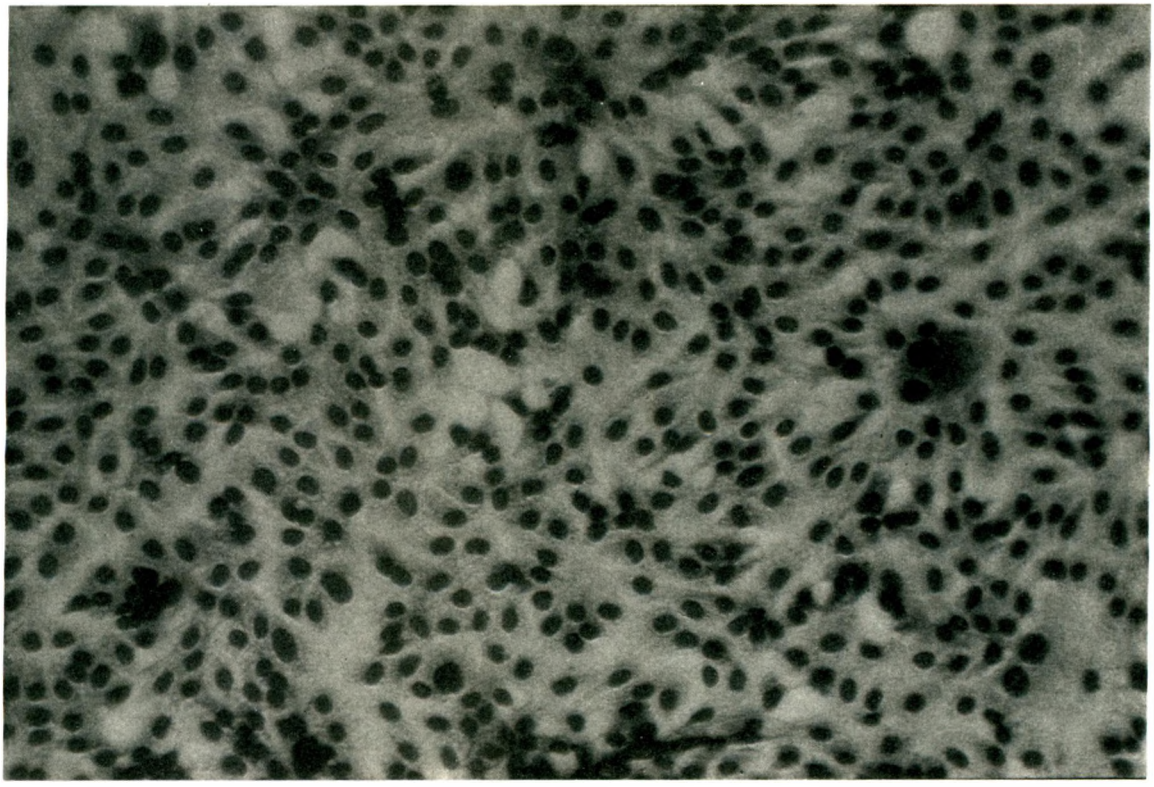


Fig.3. VETEC positive stool sample \ 10 X

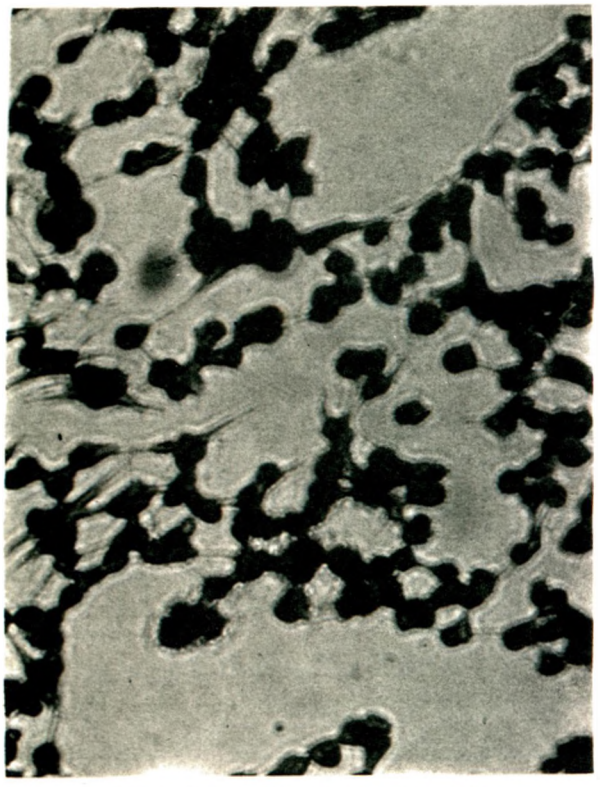


Fig.4. Standard C. difficile toxin \ 10 X

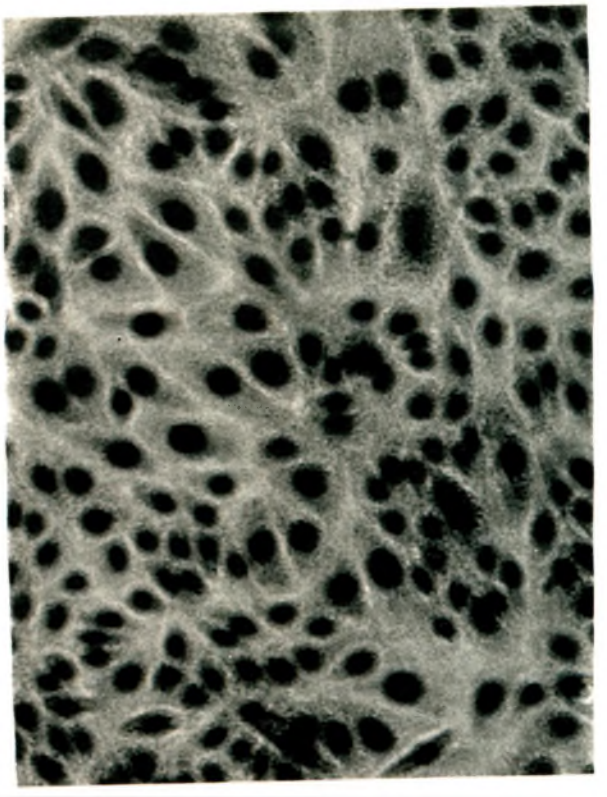


Fig.5. C. difficile toxin antitoxin neutralisation \ 10 X

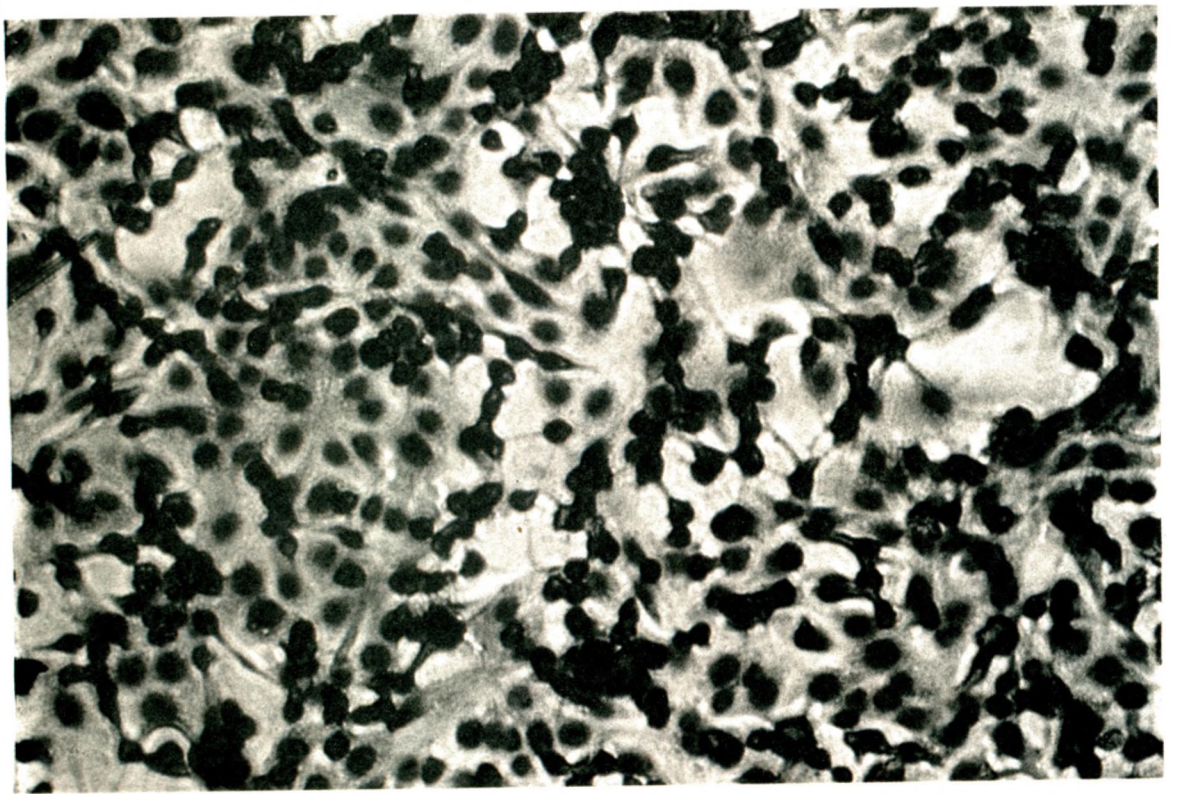


Fig.6. C. difficile toxin positive stool sample \ 10 X

DISCUSSION

The aim of the study was to determine the incidence of Aeromonas, Clostridium difficile, Yersinia enterocolitica and VETEC in addition to routinely identified pathogens and to discuss the necessity and cost effectiveness of the isolation procedures for these uncommon enteric pathogens.

Those uncommon enteric pathogens has been isolated in different rates in different geografic regions. For instance, Yersinia enterocolitica has isolation rate of 3.7% in Italy, 0.8% in Czechoslovakia, 0.5% in Spain, 0.2% in USA, 0% in Kuba (15-19). Although it has been reported in isolation rates of 0.7-1.7 in Türkiye, we hadn't any isolate during the study period (20,21). It was the same for Aeromonas spp which has been reported with an incidence of 3.3% in South Africa, 2.9% in Hawai, 1.9% in Switzerland, 0.9% in Korea and 0.3% in Yugoslavia (19,22-25). VETEC, being responsible for hemorrhagic colitis and consequent severe complications and occuring as sporadic cases or outbreaks has isolation rates of 0.5% in USA, 0.5% in England, 0.2% in Belgium (18,26,27). Only one patient (%0.2) had suffered from VETEC infection during the study period. Despite its relatively lower incidence, it should be taken into consideration in the cases of hemorrhagic colitis.

With a higher rate of isolation, particularly in hospitalised patients Clostridium difficile has an incidence of 11.1% in India, 7.8% in USA, 4.8% in Australia, 4% in England, 2.9% in Thailand (18,20,28,29,31). All Clostridium difficile isolates in our study belonged to inpatients with an isolation rate of 12.9%

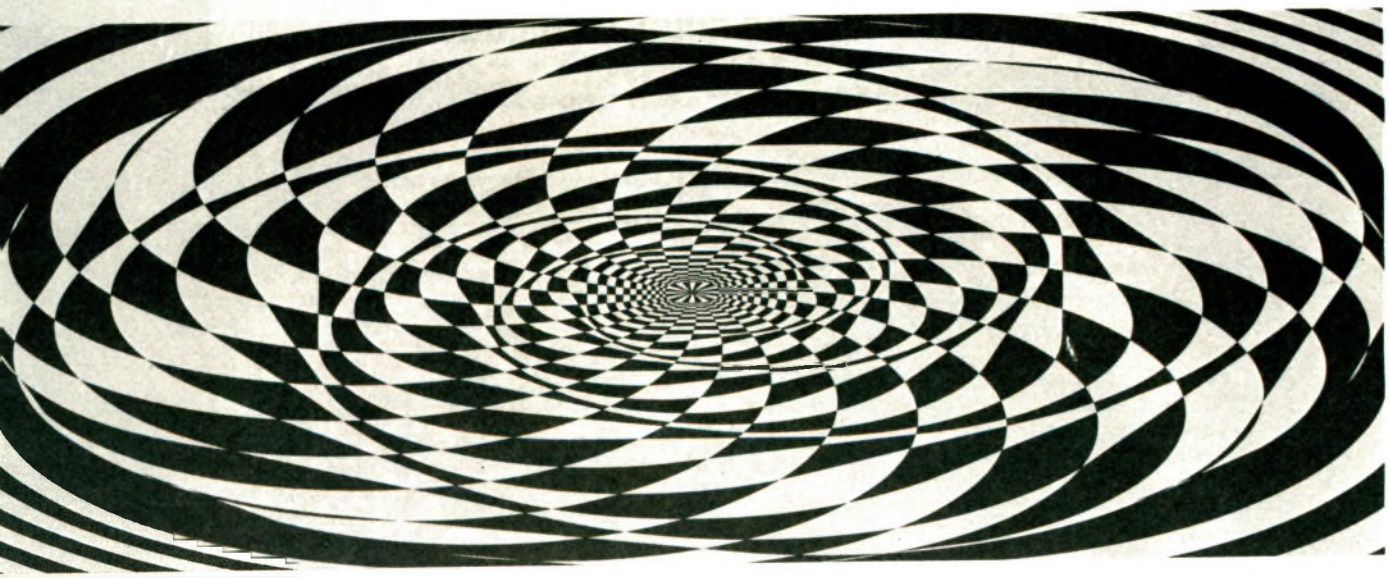
In conclusion this study indicates that routinely detectable Salmonella and Shigella strains are the commonest enteric pathogens in our country, in addition, Clostridium difficile is an important enteric pathogen for hospitalised patients. On the contrary in some countries, Yersinia and Aeromonas are isolated in comparable rates with routinely isolated pathogens whereas in our country this is not the case. The isolation procedures for this microorganisms are neither cost effective nor necessary. Since VETEC may cause life-threatening complications; isolation of this microorganism is advisable at least for reference laboratories. According to the result of this study Clostridium difficile seems to be an important pathogen as a cause of nosocomially acquired diarrhea and laboratory fascilities for determination of the toxic effects of this microorganism should be included in routine procedures at least in reference laboratories.

REFERENCES

1. *Sommers H, Stanford S. Infectious diarrhea. In: Schulman S, Pair J, Sommers H, eds. The Biologic and clinical basis of infectious diseases. Mexico: WB Saunders Company, 1992:270-293.*
2. *Guerrant G, Aravjo G, Soares E, Katloff K, Lima A.Lactoferrin: as a marker of fecal leukocytes. J Clin Microbiol 1992;30:1238-1242.*
3. *Bradley S, Tilton R, Weissfield A. Laboratory diagnosis of bacterial diarrhea. Cumitech 1980;12:1-15.*
4. *Guerrant R. Principles and syndromes of enteric infection. In: Mandell G, Douglas G, Bennett J, eds. Principles and Practice of Infectious diseases. 3rd ed. USA: Churchill Livingston Inc, 1990:837-851.*
5. *Bisset M, Powers C, Abbott S, Janda M. Epidemiologic investigation of Yersinia enterocolitica and related species: Sources, frequency, and serogroup distribution. J Clin Microbiol 1990;28:910-911.*
6. *Janda JM, Duffey PS. Mesophilic Aeromonads in human disease: current taxonomy, laboratory identification and infectious disease spectrum. Rew Inf Dis 1988;10:988-995.*
7. *Pai CH, Gordon R, Sims HV, Byran LE. Sporadic cases of hemorrhagic colitis associated with E. coli 0157:H7. Ann Intern Med 1984;101:624-627.*
8. *George RH. The carrier state: Clostridium difficile. J Antimic Chemoth 1986;18 (Supp):47.*
9. *Baron E, Finegold S. Microorganisms Encountered in the Gastrointestinal Tract. In:Baron E, Finegold S, eds. Bailey and Scott's Diagnostic Microbioloy. St Louis: Mosby Co, 1990:238-252.*
10. *Karmali M, Petric M, Corazan LM, Fleming P. The association between idiopatic hemolytic uremic syndrome and infection by verotoxin producing E. coli. J Inf Dis 1985; 151:775-780.*
11. *Dudley MV, Schotts JR. Medium for isolation of Yersinia enterocolitica. J Clin Microbiol 1979;10:180-186.*
12. *George WL. Aeromonas associated diarrhea. Clin Microbiol Newsletter 1987;9:121-125.*
13. *Marler ML, Sider J, Woolters L, Pettigrew V. Comparison of five culture procedures for isolation of C. difficile from stools. J Clin Microbiol 1987;25:1043-1048.*
14. *Lyerly DM, Krivan HC, Wilkins T. Clostridium difficile: Its disease and toxins. Clin Microbiol Rev 1988;1:18-29.*
15. *Blanco J, Ganzales EA, Blanco M. Enterotoxigenic E. coli associated with infant diarrheae in Galicia. J Med Microbiol 1991;35:162-167.*
16. *Hofstetr A, Dvoraka A. A 3 year follow up study of the incidence of Campylobacteriosis in pediatric population. Cesk Pediatr 1990;45:651-656.*
17. *Gonzales MA, Alverez JA, Mendoza MC. Epidemiological and microbiological features of Yersinia enterocolitica infections in a community during four year period. Eur J Epidemiol 1990;6:1849.*
18. *Marshall W, Mc Limans C, Yu P, Allenberger F. Results of a 6 month survey of E. coli 0157:H7. Mayo Clinic Proceedings 1990;65:787-791.*
19. *Monte Boada RJ, Perez Rodriguez M. Yersinia enterocolitica: Investigation in 1300 children under 5 years of age with acute diarrhea. Rev Cubana Med Trop 1990;42:13-18.*
20. *Candan I, Töreci K. Istanbul da gastroenteritli çocuk olgularından Yersinia enterocolitica izolasyonu ve erişkinlerde Yersinia antikorları saptanması. Infeksiyon Dergisi 1989;3:1-8.*
21. *Kayaokay Y. Akut bakteriyel gastroenteritlerde Y. enterocolitaca'nın yeri. Hacettepe ihtisas tezi 1992.*

22. Michail IA, Fox E, Heberberger RL. Epidemiology of bacterial pathogens associated with infectious diarrhea in Djibouti. J Clin Microbiol 1990;28:956-960.
23. Philips DL, Pien FD, Leong TA. Clinical features of Aeromonas enteritis in Hawai. Trans R Soc Trop Med Hyg 1990;84:126-130.
24. Desgrandchamprs D, Munzinger J. Infectious gastroenteritis in immunocompetent child. Schweiz Med Wochenesscrh 1991; 119:276-281.
25. Fridrich S, Stojkovski K. Are Aeromonas species a cause of diarrheal diseases in Yugoslavia. Vajnasanit Pregl 1989; 46:334-339.
26. Pierard D, Ettrijck V, Breynaert J, Moriau L, Results of screening for Verotoxin producing E. coli. Euro J Clin Microbiol Inf Dis 1990;9:198-202.
27. Walker CW, Upson R, Warren RE. Haemorrhagic colitis: detection of verotoxin producing 0157:H7 in a cilincal laboratory. J Clin Pathol 1988;41:80-85.
28. Miyagi SK, Bhattacharya SK, Dutta P, Naik TN. Prevalance of Clostridium difficile in hospitalised patients with acute diarrhea in Calcutta. J Diarrheal Dis Res 1991;9:16-20.
29. Mc Farland L, Surawicz, Stamn E. Risk factors for Clostridium difficile carriage and Clostridium difficile associated diarrhea in a cohort of hospitalised patients. J Infec Dis 1990;162:678-683.
30. Corrado OJ, Mascie T, Hall MJ, Bolton RP. Prevalance of Clostridium difficile one mixed function ward for the elderly. J Inf Dis 1990;21:287-291.
31. Wongawanich S, Ramsiri S, Vanasin B, Khowsaphit P. Clostridium difficile associated disease in Thailand. South Asian J Trop Med Public Health 1990;21:367-372.

POST OPERATİF EMESİS ÖNLENEBİLECEK BİR RİSK



BİLEŞİMİ: Her tablet veya ampulde 4 veya 8 mg ondansetron (hidroklorür dihitrat)

ENDİKASYONLARI: Sitotoksik kemoterapi ve radyoterapinin neden olduğu bulantı ve kusmaların tedavisi ve postoperatif bulantı, kusmaların önlenmesi ve tedavisi.

KONTRENDİKASYONLARI: İlacın bileşimindeki maddelerden birine aşırı duyarlılık durumunda kullanılmamalıdır.

UYARILAR, ÖNLEMLER: Hamilelik: Diğer ilaçlarda olduğu gibi, ondansetron, hamilelik sırasında, özellikle hamileliğin ilk üç ayında, hastaya sağlayacağı terapötik yararlar fetüse olabilecek muhtemel risklerini dengelemedikçe kullanılmamalıdır. **Emziren anneler:** Deneyler, ondansetronun sıçanlarda süt ile atıldığını göstermiştir. Bu nedenle, ondansetron alan annelerin bebeklerini emzirmemeleri tavsiye edilir.

YAN ETKİLER: Başağrısı, başta ve epigastriumda kızarma ve sıcaklık duygusu, bazen geçici, asemptomatik aminotransferaz artışları gibi yan etkiler görülebilir. Ondansetron kalın bağırsaktan geçiş süresini artırır ve bazı hastalarda kabızlığa neden olabilir. Nadiren erken aşırı duyarlılık reaksiyonları rapor edilmiştir.

POZOLOJİ: Yetişkinler: Doz günde 8-32 mg arasında değiştirilebilir. **Post-operatif bulantı ve kusma için pozoloji: Yetişkinler:** Zofran, profilaktik amaçla anesteziden 1 saat önce 8 mg oral olarak verilebilir, sonra 8 saat arayla iki kez 8 mg oral devam edilir. Veya anestezi indüksiyonunda tek doz iv. 4 mg verilir. Post-operatif bulantı kusmanın tedavisinde tek doz 4 mg iv. verilir

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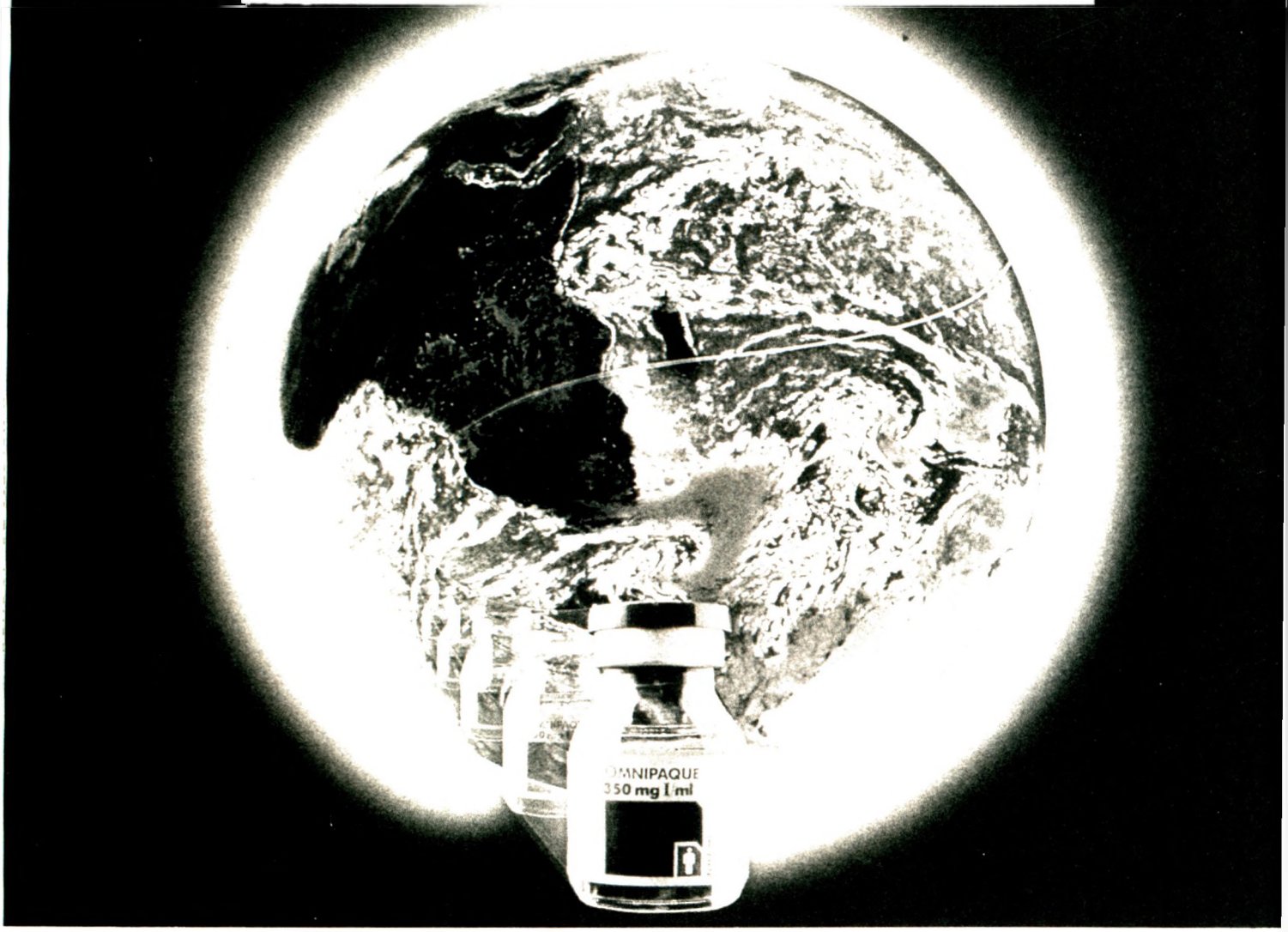

PROSPEKTÜS ÖZETİ

İntravasküler ve intratekal kullanıma uygun röntgen kontrast madde.

TANIMI: Iohexol, N, N'-bis (2,3 - dihidroksipropil) - 5 - [N- (2,3 - dihidroksipropil) asetamido] -2, 4, 6- triyodoizofitalamid molekül ağırlığı 821 (iyod içeriği % 46,36) olan triyodün, non-iyonik, suda çözünür bir kontrast maddedir.

İNTRAVASKÜLER UYGULAMA: Omnipaque ile yapılan klinik çalışmalar, bu maddenin erişkinler ve çocuklarda ürografi ve kardiyoanjiografide, sadece erişkinlerde serebral arteriografi, periferik ve çeşitli arteriografiler,flebografi, DSA ve BT tetkiklerinde intravasküler kontrast madde olarak kullanıma uygunluğunu kanıtlamıştır.

ENDİKASYONLAR: Anjiografi, ürografi, flebografi, bilgisayarlı tomografi.

UYARILAR: Allerjik natürlü hastalarda ve iyotlu kontrast maddelere duyarlılarda dikkatli olunması gerekmektedir. Bu gibi durumlarda kortikosteroid veya antihistaminik uygulanabilir.

YAN ETKİLER: Geçici ateş basması, hafif ağrı, kızarma, bulantı/kusma, hafif göğüs ağrısı ve hafif cilt reaksiyonları gibi önemsiz reaksiyonlar az sayıda hastada görülebilir.

DOZAJ VE UYGULAMA: Orijinal prospektüse bakınız.

SUBARAKNOİDAL UYGULAMA: Omnipaque lomber,torasik ve servikal miyelografiler için klinikte denenmiştir.

ENDİKASYONLAR VE UYGULAMA: Lomber, torasik ve servikal miyelografiler.

KONTRENDİKASYONLARI: Epilepsi, miyelografinin tekrarı (dozaj tablosu nedeniyle), bakteriyemi şüphesi olan belirgin lokal veya sistemik enfeksiyon mevcudiyetinde lomber enjeksiyondan kaçınılmalıdır.

YAN ETKİLER: En sık rastlanan yan etkiler başağrısı, bulantı ve kusmadır. Gözlenen diğer önemsiz yan etkiler arasında geçici baş dönmesi, sırtta, boyunda veya kol ve bacaklarda sızı yada gittikçe artan ağrı ve uyuşukluklar sayılabilir.

DOZAJ VE UYGULAMA: Orijinal prospektüse bakınız.

VÜCUT KAVİTELERİNE UYGULAMA: Omnipaque, oral kullanım dahil, çeşitli vücut kavitelerinin tetkiki için denenmiştir. Vakaların büyük bir kısmında iyi hatta mükemmel görüntüler elde edilmiştir ve Omnipaque'in çeşitli tetkikler için uygun olduğu tespit edilmiştir.

ENDİKASYONLAR: Artrografi, endoskopik retrograd pankreatografi (ERP), endoskopik retrograd kolanjiopankreatografi (ERCP), hemorografi, histerosalpingografi, sialografi ve oral suda erişen kontrast madde kullanarak gastroentestinal yolun tetkiki.

YAN ETKİLER: Omnipaque ile ortaya çıkabilecek yan etkiler diğer kontrast maddeler ile benzerdir.

DOZAJ: Orijinal prospektüse bakınız.

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