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The pathogenic detection for 126 children with diarrhea and drug sensitivity tests

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Abstract. – OBJECTIVE: To investigate the cause of infective diarrhea in children, special distribution of the infection and the drug sensitivity.

PATIENTS AND METHODS: We carried out pathogenic detection and drug sensitivity tests for one hundred and twenty-six children with diarrhea, including 67 children in infant group (0-<1 year) and 59 children in child group (1-5 years).

RESULTS: Fat particles and starch particles were seen in most stool samples under microscope; 42 kinds of pathogenic bacteria were obtained from 126 stool samples, including 10 Diarrheagenic *E. coli* (8 *Enteropathogenic E. coli* and Enteroinvasive E. coli); there were found 53 positive samples in the detection of anti-rotavirus antibody in 126 stool samples; in the drug sensitivity tests for major pathogenic bacteria, *Staphylococcus aureus*, *Shigella, Salmonella* and Diarrheagenic *E. coli* were highly sensitive.

CONCLUSIONS: The abuse of antimicrobial agents may result in intestinal disorders, leading to aggravation in diarrhea and antimicrobial agents based on drug sensitivity tests may be served as fundamental treatment of diarrhea for children.

Key Words:
Diarrhea, Drug sensitivity test, Pathogenic bacteria, Stool.

Introduction

Diarrhea in children is divided into infective diarrhea and non-infective diarrhea, and it includes a series of clinical syndromes of diarrhea caused by multiple pathogens and factors. Infective diarrhea is usually caused by intestinal tract infection of viruses, bacteria, fungi and parasites. It is a common and prevalent disease in children, and a major cause of infant and child death. Common pathogenic microorganisms include viruses (rotavirus, adenovirus, etc.), bacteria (pathogenic *Escherichia coli*, *Shigella Dysenteriae*, and *Salmonella*, etc.), protozoa and fungi[1]. Currently, among the 12 million children aged less than 5 years who have died each year, 3-4 million are caused by diarrhea. Diarrhea is a common and prevalent disease only second to respiratory infection[2-4].

Patients and Methods

Patients

This study was approved by the Ethics Committee of Xuzhou Children's Hospital. Signed written informed consents were obtained from the guardians all participants before the study.

One hundred and twenty-six children with diarrhea (0-5 years old) who were treated in this hospital from October 2011 to December 2012 were included in this study. There were 67 children in infant group (0-<1 year) and 59 children in child group (1-5 years). Subjects were those patients with diarrhea who defecated loose stools ≥ 3 times within 24 h and 72 h or longer[5].

Detection Method

Regular stool examination: appearance, leukocytes, erythrocytes, crystal, fat particles, starch particles, parasite egg and protozoa. Stool culture: stool sample was inoculated on *Salmonella* and *Shigella* (SS) medium plate for isolation of *Salmonella* and *Shigella*, MacConkey medium plate (MacC, for the culture of Gram-negative bacteria), Sabouraud agar medium plate (for the characterization and pure culture of fungi) and blood agar medium plate (for the culture of *Enterococcus*); pure culture and automatic biochemical characterization were performed for suspicious pathogenic bacteria; pure culture of *Salmonella*, *Shigella* and *Escherichia coli* (*E. coli*) were subject to corresponding serologic identification, and drug-sensiti-

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vity analysis was performed with drug-sensitivity plates for pathogenic bacteria. The identification of Diarrheagenic *E. coli*: Single colony of *E. coli* was used in slide agglutination test with 3 multivalent anti-O sera (Enteropathogenic *E. coli*, Enteroinvasive *E. coli*, Enterotoxigenic *E. coli*) and EHEC O157 diagnosis serum, no agglutination was reported as negative. If agglutination occurred with one multivalent anti-O serum, the test should be verified with each monovalent anti-O serum included in the multivalent anti-O serum, agglutination was reported as positive. The serological identification of *Salmonella*: slide agglutination test was performed with A-F multivalent anti-O sera. If agglutination occurred, identified the O group of each detected bacterium with the anti-O sera representing 6 O groups. The serological identification of *Shigella*: mixed the bacteria with 4 multivalent anti-*Shigella* sera, visible granular aggregation within minutes was determined to be positive. Then, the monovalent sera of A, B, C and D group were used to identify the bacteria. The detection of rotavirus in stool: double antibody sandwich immunochromatography was used for the detection of rotavirus. The detection was performed according to the instructions.

Instruments and Reagents

Olympus high power microscope (Olympus, Tokyo, Japan), automatic bacteria characterization/drug-sensitivity system (VITEK 2Compact) ATB expression and the relevant reagents were provided by bioMeriéux Co (Marcy L'Etoile, France). Culture medium was purchased from Beijing Luqiao Technology Inc. All typing and characterization sera were purchased from Lanzhou Biological Products Institute. Rotavirus was purchased from Beijing Wantai Biological Pharmaceutical Co., Ltd.

Statistical Analysis

SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Chi-square test was used for the comparison of positive rate. α=0.05 was set as the inspection level, and p<0.05 indicated the difference had statistical significance.

Results

Regular Stool Examinations of 126 Stool Samples

As shown in Tables I and II, the stool in infant group was predominantly green and egg-drop shaped; the stool in child group was predominantly yellow, watery and loose; fat particles and starch particles were seen in most stool samples via microscopy, in which ascaris eggs were detected in 1 sample.

The Culture of Pathogenic Bacteria in Stool

As shown in Table III, we obtained 42 kinds of pathogenic bacteria from 126 stool samples, including 10 Diarrheagenic *E. coli* (8 Enteropathogenic *E. coli* and Enteroinvasive *E. coli*), 13 Gram-positive cocci (8 *Staphylococcus aureus* and 5 conditional pathogenic cocci), 5 *Candida albicans*, *Shigella* (5 B-group *Shigella flexneri*, 2 D-group *Shigella sonnei*, 4 *Salmonella* (3 *Salmonella choleraesuis* and 1 *Salmonella paratyphi* B). In the stool with pathogenic bacteria after culture, 32 cases of abnormal leukocyte and 7 cases of abnormal erythrocyte were cultured from mucosal stool or bloody purulent stool, and positive results were seen simultaneously in pathogenic bacterial culture and detection of rotavirus.

Detection of Rotavirus in Stool

As shown in Table IV, the detection of anti-rotavirus antibody in 126 stool samples revealed 53 positive samples, and the positive rate was 32.84% and 52.54% in infant group and child group, respectively, and the difference was statistically significant (χ^2=4.99, p=0.025). Among 53 samples with positive results in detection of rotavirus, sto-

Table I. The color and appearance of stool in regular stool examination.

		Color				Appearance				
Group	No.	Yellow	Green	Red	Other	Loose	Watery	Bloody purulent	Mucosal	Egg-drop
Infant	67	20	41	0	6	11	15	0	10	31
Child	59	33	11	8	7	21	18	9	6	5
In total	126	53	52	8	13	32	33	9	16	36

Table II. Abnormality in regular stool examination (no.).

Group	No.	Leukocytes	Erythrocytes	Fat particles	Starch particles	Parasitic eggs	Occult blood test	Residual crystals
Infant	67	14	0	35	29	0	1	18
Child	59	18	9	21	12	1	8	36
In total	126	32	9	56	41	1	9	54

More than 3 leukocytes and erythrocytes were considered abnormal; ≥1+ (5 events in one high-amplification field were considered 1+) fat particles, starch particles and residual crystals were considered abnormal.

Table III. The culture of pathogenic bacteria in stool samples.

Culture	Infant group (no.=67)	Child group (no.=59)	In total
Diarrheagenic *E. coli*	4	6	10
Gram-positive cocci	7	6	13
Candida albicans	2	3	5
Shigella	0	7	7
Salmonella	3	4	7
Normal flora growth	51	33	84

Table IV. The statistical analysis of the regular stool examination of the stool with positive detection of rotavirus.

Group	No.	Positive rate [no. (%)]	Appearance		Color		
			Watery	Others	Green	Yellow	Others
Infant	67	22 (32.84)	12	10	14	6	2
Child	59	31 (52.54)	16	15	10	18	3

ol was predominantly watery, and the color was predominantly yellow or green.

Drug Sensitivity Test

As shown in Table V, in the drug sensitivity tests for major pathogenic bacteria, *Staphylococcus aureus*, *Shigella*, *Salmonella* and Diarrheagenic *E. coli* were highly sensitive to imipenem, amikacin, aztreonam, piperacillin ciprofloxacin and levofloxacin, and less sensitive to cephalosporins aminoglycosides.

Discussion

Regular stool examinations of 126 samples showed that diarrhea in children led to predominantly loose stool, watery stool and egg-drop stool, with yellow and green color. Leukocytes and erythrocytes were scarcely seen; instead, fat particles and starch particles were predominant. Inspectors are required to be aware of common appearance and regular microscopy pattern of the stool in children with diarrhea. Ascaris eggs were found in 1 sample from village. Although the hygiene condition had been improved, parasitic infection still occurred in some regions with poor hygiene condition, which requires more attention from medical staff and inspectors. With the popularization of stool station, inspectors should master the detection methods of common parasites and the morphology of parasite eggs, and pay more attention to stool microscopy.

Rotavirus is the most important pathogen leading to the diarrhea in children aged less than 5 years[6]. In this study, 53 positive cases of anti-rotavirus antibody were detected in 126 stool samples, which was consistent with previous reports[7-9]. The positive rate in infant group (32.84%) was lower than child group (52.54%) ($p<0.05$), which may be associated with infant's diet. Infants are predominantly fed by breast milk, but with an increase in the activity of child, the anti-rotavirus antibody will diminish gradually, leading to increased risk in infection of rotavirus. Regular stool examination of the rotavirus-infected patients showed

Table V. Drug sensitivity of major pathogenic bacteria in stool culture to common antimicrobial agents (%).

Antimicrobial agents	Shigella	Diarrheagenic *E. coli*	Salmonella	*Staphylococcus aureus*
Levofloxacin	70.00	54.95	95.87	79.00
Gentamicin	50.00	32.97	65.65	68.66
Amoxicillin	10.00	37.36	66.60	32.05
Piperacillin	100.00	63.74	30.45	87.10
Imipenem	100.00	95.60	85.25	37.97
Ciprofloxacin	77.90	49.45	66.38	74.00
Cefotaxime	22.80	9.89	63.54	35.85
Cefoxitin	55.80	58.24	66.42	36.54
Linezolid	100.00	-	48.25	-
Amikacin	100.00	87.50	65.25	-
Quinupristin	91.50	-	-	89.55
SMZ-TMP	30.00	36.90	30.00	73.13
Vancomycin	100.00	-	-	97.00
Aztreonam	20.00	77.90	100.00	-
Ampicillin/ Sulbactam	0	5.49	15.50	5.00
Cefazolin	5.50	9.80	85.00	32.52
Ceftriaxone	10.00	15.70	75.66	25.36
Cefepime	1.70	16.30	73.00	32.01
Tobramycin	-	55.80	-	-
Erythromycin	-	-	-	20.90
Clindamycin	-	-	-	40.50
Penicillin	0.00	3.30	25.00	5.00

-: N/A for drug sensitivity test

that the stool was predominantly green, yellow and watery stool, fat particles and starch particles were more common, whereas erythrocytes and leukocytes were less seen. This may be caused by the fact that immunologic injury of intestinal mucosa led to poor intestinal absorption, which gave rise to the above-mentioned symptoms. Therefore, the detection of rotavirus is suggested for similar stool.

In this study, the infection of pathogenic positive cocci and Diarrheagenic *E. coli* was different from the previous reports[10-11], which showed that diarrhea in children was predominantly due to pathogenic *E. coli*, and secondly rotavirus infection. It is possible that the living habits in different regions cause a different kind of pathogenic infection, and an increasing trend has been seen in rotavirus infection. This study demonstrated that the incidence rate of *Shigella* and *Salmonella* infection would increase with age. Increased outdoor activities with age may lead to gradually complicated bacterial infection. Previous studies reported that *Shigella* is the most dominant pathogenic bacteria, followed by *Salmonella* and pathogenic *E. coli*[12]; Behiry et al[13] reported that pathogenic *E. coli* is the dominant pathogenic bacteria for diarrhea in children.

Pathogenic bacteria were cultured in 3 stool samples with detection of rotavirus. Due to rotavirus infection with bacterial infection, both anti-viral and anti-bacterial treatment should be considered in these conditions. This study found 5 cases of Gram-positive opportunistic pathogenic bacteria infection and 5 cases of fungal infection, and all cases had flora imbalance due to high dosage of antimicrobial agents before or compromised immunity, leading to delayed chronic diarrhea, which should be taken seriously. Therefore, fungal infection in intestinal tract should not be neglected. Intestinal fungal infection predominantly occurs in elderly children. Therefore, the abuse of antimicrobial agents may cause flora imbalance and fungal proliferation in the intestinal tract, leading to diarrhea[14].

Conclusions

In the drug sensitivity tests of major pathogenic bacteria, Staphylococcus aureus, *Shigella*, *Salmonella* and *Diarrheagenic E. coli* were highly sensitive to imipenem, amikacin, aztreonam, piperacillin ciprofloxacin and levofloxacin, but less sensitive to aminoglycoside cephalosporin. This was similar to previous reports[15]. The abuse of antimicrobial agents may result in intestinal disorders, leading to aggravated diarrhea. It is sug-

gested to choose antimicrobial agents based on drug sensitivity tests in the treatment of diarrhea in children due to bacterial infection[16].

Conflict of interest

The authors declare no conflicts of interest.

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