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Prevalence of Bacteria in the Nationwide Survey of Stool Culture Performed in 2015, Korea

Won-Hee Choi[1]*, Jung-Hyun Byun[2]*, Sunjoo Kim[2]

[1]Department of Nursing Science, Kyungsung University, Busan, [2]Department of Laboratory Medicine, Gyeongsang Institute of Health Sciences, Gyeongsang National University School of Medicine, Jinju, Korea

Background: The spectrum of bacteria causing diarrhea is highly affected by geographic area, sanitation, travel, food consumption, and previous antibiotic use. A nationwide databank for stool cultures is undeveloped. The aim of our study was to investigate the current prevalence of gastroenteritis bacterial pathogens in Korea.
Methods: We requested microbiological data via questionnaire emails sent to 98 hospitals. The frequency of each pathogen was acquired from 32 institutes. Numbers of stool cultures performed ranged from 193 to 14,296 (mean 2,724, SD 3,261) in 2015.
Results: Among 86,744 requested stool specimens, 917 (1.06%, range 0-4.59%, 95% confidence interval 0.63-1.48%) were positive. *Salmonella* was most prevalent (59.0%), followed by *Candida* (12.4%), *Campylobacter* (4.8%), *Staphylococcus aureus* (4.0%), *Vibrio* (4.0%), and *Pseudomonas aeruginosa* (1.75). *Yersinia* (0.3%) and *Shigella* (0.2%) were rarely isolated.
Conclusion: As the positive rate of the stool cultures is very low (1.06%), more effort and concern should be provided to enhance the isolation of pathogens. *Salmonella* was the most prevalent pathogen and *Campylobacter* and *Vibrio* were relatively common pathogens causing bacterial gastroenteritis in Korea. **(Ann Clin Microbiol 2016;19:105-109)**

Key Words: *Campylobacter*, Diarrhea, Prevalence, *Salmonella*, *Vibrio*

INTRODUCTION

Gastrointestinal infection is one of the most common illnesses and is a leading cause of death in young children in developing countries [1-3]. Viruses such as rotavirus, norovirus, adenovirus, and astrovirus, as well as parasites such as *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium*, cause diarrhea in many regions worldwide. *Clostridium difficile* is arising as a common pathogen especially in those groups receiving antibiotics in developed countries [2-4]. Common bacteria causing gastrointestinal infection in the community are *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio*, pathogenic *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus cereus*, and *Yersinia* [2,3,5]. The prevalence of these viruses, parasites, or bacteria causing diarrhea is highly affected by age, living area, sanitation, travel, cooking, and food consumption [6]. The spectrum of pathogens is also different by manifestation of diarrhea, that is, dysentery, traveler's diarrhea, watery diarrhea, nosocomial diarrhea, persistent diarrhea, or food poisoning [2,3,5,7]. Nationwide data of stool culture is scarce so far. The aim of our study was to investigate the prevalence of common bacterial pathogens causing gastroenteritis in 2015, in Korea.

MATERIALS AND METHODS

Ninety-eight institutes, where clinical microbiology laboratories are located, were requested to submit the annual data of stool cultures performed in 2015. If the specimen numbers are less than 100, or stool cultures were performed outside of the institutes, those data were excluded. Among them, 32 institutes submitted the adequate data. Any microorganisms of which clinical significance is not known were included for the analysis.

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Correspondence: Sunjoo Kim, Department of Laboratory Medicine, Gyeongsang National University Hospital, 79 Gangnam-ro, Jinju 52727, Korea. (Tel) 82-55-214-3072, (Fax) 82-55-214-3089, (E-mail) sjkim8239@hanmail.net

*These authors contributed equally to this work.

Average stool culture numbers were 2,711 (SD 3,261, range 193-14,296).

RESULTS

Thirty two institutes who submitted adequate data included 1 referral laboratory, 17 (53.1%) tertiary care hospitals. Twelve institutes are located in Seoul, 7 in Gyeonggi province, 7 in Gyeonsang province including 3 in Busan, 3 in Jeolla province, 2 in Chuncheong province, 1 in Jeju province. Five institutes have less than 300 beds, 18 between 300-1,000 beds, 6 between 1,001-2,000 and 2 more than 2,000 beds.

Among 86,744 stool specimens performed for culture in 2015, 917 (1.06%, 95% confidence interval 0.63-1.48%) yielded positive isolates. Among 917 isolates, *Salmonella* constituted 59.0%, followed by *Candida* (12.4%), *Campylobacter* (4.8%), *Vibrio* (4.0%), *S. aureus* (4.0%), *Klebsiella pneumoniae* (1.6%), *Pseudomonas aeruginosa* (1.5%), and *B. cereus* (1.3%) (Table 1). *Yersinia* (0.3%), and *Shigella* (0.2%) were rarely isolated. One isolate of *Plesiomonas shigelloides*, *Shewanella putrefaciens*, and *Enteroccus faecium* was reported as positive. In addition, four institutes submitted the positive rate of *C. difficile*, which is 9.0% (1,571/17,495).

Table 1. Frequency of microorganisms isolated from stool culture in 2015 compared with the previous study analyzed in a university-affiliated hospital

Bacterial isolates	Cho et al. (2001-2010) [9]		This study (2015)	
	N	%	N	%
Salmonella	359	63.0	541	59.0
Salmonella spp.	2		64	
Salmonella Typhi	1		3	
Serogroup A	2		1	
Serogroup B	86		191	
Serogroup C	89		137	
Serogroup D	157		129	
Serogroup E	22		14	
Shigella	98	17.2	2	0.2
Shigella flexneri			1	
Shigella boydii			1	
Campylobacter	85	14.9	44	4.8
Campylobacter jejuni			42	
Campylobacter coli			2	
Vibrio	27	4.7	37	4.0
Vibrio parahaemolyticus	27		26	
Other *Vibrio* spp.			11	
Yersinia	1	0.2	3	0.3
Yersinia pseudotuberculosis			1	
Yersinia enterocolitica	1		2	
Others*			190	20.7
Total	570	100.0	917	100.0

**Candida* 114, *Staphylococcus aureus* 37, *Pseudomonas aeruginosa* 14, *Bacillus cereus* 12, *Klebsiella pneumoniae* 10, *Plesiomonas shigelloides* 1, *Shewanella putrefaciens* 1, *Enterococcus faecium* 1.

DISCUSSION

This is a nationwide survey of stool cultures performed in 2015 from 32 institutes, of which a positive rate was very low (1.06%). Lee [8] reported the positive rate of stool culture as 2.6% in a tertiary care hospital during 5 years. Cho et al. [9] reported that the positive rate was decreasing compared with the past decades: 12.9% in the 1970s vs. 1.1% in the 2000s. Yields of stool culture abroad were reported as also low from 1.5% to 2.9% [3]. Laboratory factors, such as inoculation within 2 h, storage in the refrigerator if transport is delayed, agar plates used, enrichment broth, technical expertise, as well as physician's awareness of diagnosis of diarrhea might affect the positive rate [3,10]. In addition, longer hospitalization for more than 3 days and previous antibiotic usage might reduce the positive rate of routine stool culture [3,10].

Salmonella requires large inoculation to cause illness, because this organism is susceptible to gastric acid [2]. In this study, *Salmonella* demonstrated to be the predominant pathogen causing diarrhea. The pathogens causing typhoid fever, such as *Salmonella* Typhi or *Salmonella* Paratyphi, seem very rare at present [9,11]. Instead, serogroup B was most common, which correlates with a previous report [8]. *Salmonella* serogroup D was most frequent in children during 1998-2008 [11,12]. In a long-term observation of stool cultures in a tertiary hospital, *Salmonella* serogroup B was predominant from 1979 to 1998, whereas *Salmonella* serogroup D exceeded other serogroups in the 2000s [9].

Recently, *Shigella* seems very rare in stool culture [9]. There were only seven reported cases of *Shigella* infection in 10 years if an outbreak was excluded in a longitudinal study [9]. As *Shigella* is easily mortal and small inoculum can cause gastrointestinal illness [2], more careful handling of the stool specimen is necessary when this organism is suspected. The stool specimens should be inoculated within 2 h [10], and buffered glycerol saline should be considered for transport [2,10]. As Selenite-F liquid enrichment broth inhibits *Shigella*, gram-negative broth may be alternatively used when *Shigella* is suspected [10].

Campylobacter inhabits chickens. Therefore, undercooked chickens or contaminated eggs are common sources of *Campylobacter* infections [2]. Although this is known as the second or third most common cause of diarrhea in the developed countries [5,9], many laboratories do not have the facility to grow this organism in Korea. It requires special selective media, microaerophilic atmosphere, and a 42°C incubator [10]. Procedures of identification of *Campylobacter* seem more complicated than those for conventional bacteria. Although only 53.1% (17/32) had the facility to grow *Campylobacter*, prevalence of *Campylobacter* was the second followed by *Salmonella*. Characteristic 'S'-shaped curved morphology in the microscopic examination might give a hint for this organism [2,10].

Vibrio was accounted for 4.0%. *Vibrio parahaemolyticus* was most common (26 isolates), followed by *Vibrio fluvialis* (four isolates) and *Vibrio alginolyticus* (one isolate). *Vibrio* is required to grow when seawater temperature is elevated in the summer. Many laboratories used thiosulfate-citrate-bile salts-sucrose (TCBS) agar during May-October in the survey (data not shown). As *Vibrio* is halophilic, addition of salt is needed for the isolation and identification of this organism [10].

Yersinia is known to cause symptoms similar to acute appendicitis; the prevalence was not high in a previous study [13]. Among 110 patients who had right lower quadrant pain, only two showed antibody responses to *Yersinia*.

Although clinical significance of *Candida*, *S. aureus*, and *P. aeruginosa* in stool culture is not clear, they might be reported to the clinicians when they are predominantly grown in the stool culture [10]. They may prevail when antibiotics suppress normal flora in the gastrointestinal tract. Sometimes they may cause antibiotic-associated diarrhea [10,14]. Consensus whether to report these organisms and to unveil the clinical significance might be needed.

Incidence of our study is quite different from the data released from Korea Centers for Disease Control and Prevention (KCDC). In the report of KCDC, *S. aureus* (5.99%) was most common, followed by pathogenic *E. coli* (4.22%) and *Salmonella* spp. (2.69%) during the same period [15]. KCDC deals with rather bloody or watery stool in a limited number of hospitals or referred from the clinics. Most laboratories in the hospital did not have the facility to identify food poisoning *S. aureus*, or pathogenic *E. coli*, *B. cereus*, and *C. perfringens*. Target organisms and culture facility between hospital laboratories and public health institute are somewhat different.

P. shigelloides was isolated only once and *Aeromonas* was not isolated. These pathogens seem very rare [9], or would be difficult to grow in the routine culture [16]. Addition of blood agar plate (BAP) might be helpful to isolate these organisms [2,10]. There were 10 isolates of *K. pneumoniae* reported in the stool cultures in our study. Although *K. pneumoniae* is a normal flora in the stool, sometimes it may cause diarrhea [2,10,14].

Although only four institutes submitted the positive rate of *C. difficile*, it accounts for 9.0% (1,571/17,495). Prevalence of *C. difficile* seems much higher than that of conventional bacteria causing diarrhea. Our data correlates with previous reports [13,17,18]. As *C. difficile* is spore-forming bacteria and inhabits the hospital environment, it seems quite difficult to eradicate this organism [4].

There are several limitations in our study. Although the authors tried to collect more data from many hospitals, it was quite difficult to analyze annual data for each institute. Many laboratories have the facility to isolate *E. coli* O157, but there was no isolate reported. These data were collected from rather big hospitals; therefore, they do not demonstrate the real frequency of bacterial gastroenteritis in the community. Also, data of viruses or parasites causing diarrhea are absent in this study. As we counted only the stool specimens, we do not know whether the stool cultures were repeatedly ordered or not. The numbers of stool cultures are quite variable by each institute, suggesting a bias by data of several large hospitals. Data of antibiotic susceptibility are omitted.

In conclusion, we collected nationwide data of stool cultures performed in 2015 from 32 hospitals. The yield (1.06%) of stool culture was very low. Therefore, more efforts to enhance isolation of etiological bacteria in conventional stool cultures, as well as adoption of new sensitive molecular techniques, are needed. *Salmonella* was most common, followed by *Campylobacter* and *Vibrio*. *Shigella* and *Yersinia* were very rare. A consensus whether to report a predominant growth of *Candida*, *S. aureus*, and *P. aeruginosa* in the stool culture is needed.

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=국문초록=

2015년 국내 의료기관 조사에서 파악된 대변배양에서 세균의 분리 빈도

[1]경성대학교 간호대학, [2]경상대학교 의과대학 진단검사의학교실, 건강과학연구원

최원희[1], 변정현[2], 김선주[2]

배경: 설사를 일으키는 세균의 종류는 주거지역, 공중위생, 여행, 음식 및 항균제 사용 여부에 따라 달라질 수 있다. 그동안 대변배양에 대한 전국적 조사는 매우 미흡하였다. 본 연구에서는 2015년 의료기관에서 흔히 분리되는 세균의 빈도를 전국적으로 조사하였다.

방법: 의료기관 98곳에 설문지를 보내어, 그 중 회신이 온 32기관의 자료를 종합하였다. 연간 대변배양 건수는 평균 2,724건(표준편차 3,261, 범위 193-14,296건)이었다.

결과: 대변배양이 의뢰된 86,744건 중 917건(1.06%, 95% 신뢰구간 0.63-1.48%)에서 양성을 보였다. 균주별로는 *Salmonella*가 59.0%로 가장 흔하였고, *Candida* (12.4%), *Campylobacter* (4.8%), 황색포도알균(4.0%), *Vibrio* (4.0%), 녹농균(1.5%) 순이었다. *Yersinia* (0.3%)와 *Shigella* (0.2%)는 매우 드물게 분리되었다.

결론: 전국적으로 대변배양 균주 분리율은 1.06%로 매우 낮으므로 분리율을 높이기 위한 관심과 노력이 필요하다. 설사의 원인균으로는 *Salmonella*가 가장 흔하였고, *Campylobacter*와 *Vibrio*는 비교적 자주 분리되었다. [Ann Clin Microbiol 2016;19:105-109]

교신저자 : 김선주, 52727, 경남 진주시 강남로 79
경상대학교병원 진단검사의학과
Tel: 055-214-3072, Fax: 055-214-3089
E-mail: sjkim8239@hanmail.net