부산광역시 보건환경연구원보 제12권, 38~47(2002)
Rep. Busan Inst. Health & Environ. Vol.12, 38~47(2002)

Molecular epidemiological characteristics of *Shigella flexneri* strains Isolated in Busan during the Period 1998 to 2002 : Antibiogram, Plasmid Profile and Serotype Correlation

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1998~2002년 부산지역에서 분리된 *Shigella flexneri*의 분자역학적 특성 : Antibiogram, Plasmid Profile, Serotype의 상관관계

미생물과

민상기 · 박은희 · 김미희 · 박지현 · 박연경 · 진성현 · 빈재훈 · 이상훈

Abstract

Thirty-three isolates of *Shigella flexneri* were obtained from patients with diarrhea during 1998 to 2002 in Busan, South Korea. To compare for typing *Shigella flexneri* we use three different epidemiological tools, serotype, antibiogram, plasmid profile. The prevalent serotypes were 2a(28 strains of the isolates), 3a(2 strains) and 2b, 5a, variant Y(1 strain respectively). All but one isolates which was susceptible to all antimicrobials tested were resistant to multiple antibiotics, particularly the first-line treatment drugs such as carbenicillin, streptomycin, chloramphenicol, tetracycline, ampicillin, ticarcillin. Almost all strains isolated were susceptible to gentamicin, kanamycin, ceftriaxone, ceftazidime, cefoxitin, ciprofloxacin. All but two isolate from which any plasmid were not detected harboured at least three plasmid(range 3-7) and exhibited 11 distinct plasmid profile pattern. We could not find specific correlation between serotype, antimicrobial resistance and plasmid profile.

Key words : *Shigella flexneri*, serotype, antimicrobial resistance, plasmid profile

INTRODUCTION

Shigella species are commonly pathogenic to humans, causing severe gastroenteritis (bacillary dysentery). In healthy adults, dysentery is a self-limiting disease, but it can be fatal to infants and young children.

Worldwide, it is estimated that shigellosis cause around 600,000 deaths per year, two-thirds of the deceased being children under 10 years old.

The disease is highly contagious due to its low infectious dose as few as 10 to 100 organisms[10]. Epidemics usually occur in areas with crowding and poor sanitary conditions, where transmission from person to person is common, or food or water is contaminated by the organism[19,20].

It is known that *Shigella dysenteriae* and *Shigella flexneri* are the predominant species in the tropics, while *Shigella sonnei* is in industrialized countries.

In Korea, *Shigella sonnei* is the most prevalently isolated species (85.76%) followed by *Shigella flexineri* (10.7%), *Shigella dysenteriae* and *Shigella boydii* (respcetively 1.7%) by 2002.

Effective antimicrobial therapy can reduce both severity and duration of illness and can prevent potential complications.

However, today *Shigella spp.* have progressively become resistant to most of the widely used and inexpensive antimicrobials over the past decades, resulting in treatment failure in Korea.

High frequencies of resistance in *Shigella flexineri* to many of the first-line antimicrobial agents have been reported in recent years from many parts of the world.[1,2,4,5,6,7,18]

However, we believe that there are no published data on the incidence of antimicrobial resistance amongst *Shigella flexineri* in Busan, South Korea.

Also the study of epidemiologic markers is important in an attempt to trace the source of infection[8].

In this work we try to deter-

minate the clonal relationship with epidemiologic typing markers such as serotype, antimicrobial susceptibility, plasmid pattern among 33 *Shigella flexineri* strains isolated from patients' feces with acute diarrheal disease during 1998 to 2002 in Busan, South Korea.

MATERIALS AND METHODS

Bacterial isolates

During 1998 througth 2002, stool specimens from patients with diarrhea were collected and screened for *shigella* species by conventional bio-chemical methods in our laboratory. Of which were recovered 259 *S. sonnei*, 33 *S. flexneri* and 2 *S. boydii* respectively.

Of the non-replicate 33 *S. flexneri* strains which were isolated from sporadic case except two epidmio-logically related strains, 6 were isolated during 1998, 20 were isolated 1999, 2 were isolated 2000, 3 were isolated 2001 and 2 were isolated during 2002 respectively. *S. flexneri* strains were routinely grown at 37℃ in Trypticase soy broth and LB broth was used to grow bacteria for plasmid DNA extraction.

Serotyping of *S. flexneri*

Shigella species recovered were serogrouped with polyclonal antiserum and serotyped with commercially available group factor and type specific antisera of *S. flexneri* (Denka Seiken Co.LTD, Tokyo, Japan) by slide agglutination test.

Antimicrobial susceptibility

Susceptibility testing was performed by an agar diffusion disk method as recommended by the National Com-mittee for Clinical Laboratory Stan-dards.[14)]

The 21 antimicrobial agent used in this study were nalidixic acid, tobramycin, gentamicin, trimethoprim/sulfamethoxazol, cepalothin, colistin, carbenicillin, streptomycin, kanamycin, amikacin, ceftriaxone, chloramphenicol, ceftazidime, ciprofloxacin, amoxicillin/clavulanic acid, cefoxitin, tetracyclin, cefotaxime, ampicillin, ceptazidime, ampicillin/sulbactam, ticarcillin and obtained from BBL Microbiology Systems(Cockeysville, Md.).

Standard control strain of *Escherichia coli* ATCC 25922 were used for monitoring the accuracy and precision

of disk diffusion test.

Plasmid profile analysis

Plasmid DNA was extracted from overnight bacterial cultures with commercial kit Wizard Plus Minipreps DNA purification system(Promega, Madison, USA) according to the manufacture's instructions.

The plasmids obtained were separated by electrophoresis on 0.5% agarose gel containing 0.5ug of ethidium bromide per ml in Tris-acetate buffer and visualized under UV light. Plasmid size were determined with 1Kb DNA ladder(Bioneer, Daejeon, Korea) and λHindIII digest (Promega, Madison, USA).

Only small plasmids, which are below 20kb molecular weight were used in the analysis. Large plasmids were not further investigated because of their instability[16]. Plasmid pattern analysis was found to be a useful epidemologic marker in an attempt to trace the source of infection.[12,17]

RESULTS AND DISCUSSIONS

S. flexneri serotypes

There are currently six established serotypes of *S. flexneri*(type 1 to 6) and two variants. Of the 33 *S. flexneri* isolates, 2a was the most prevalent serotypes (84.8%) and 3a (6.0%), 2b, 5a, variant Y(3.0%, respectively) were followed in order.

Antimicrobial resistance patterns

The antimicrobial resistance phenotype of each isolates is shown in the Table 1. With the exception of one strain isolated in 1998 which were sensitive to all of antimicrobials, all the other isolates were resistant to at least six drugs and supposed to be multiply drug resistance. Two strains of the 33 strains were resistant to nine antibiotics (phenotype spectra Ⅷ, Ⅸ; see table 1. for phenotype spectra), three strains to eight (Ⅵ, Ⅶ), ten strain to seven(Ⅳ, Ⅴ), 17 strains to six(Ⅱ, Ⅲ).

Among the 9 antibiotic resistance spectra detected, two predominated, i.e. Cb,S,C,Te,Am,Tic and SXT,Cb, S,C,Te,Am,Tic resistance spectra (50.0 and 25.0 percent of the resistance strains respectively).

All 32 isolates were resistant to carbenicillin, streptomycin, chloramphenicol, tetracycline, ampicillin, ticarcillin (100%), 13 to trimethoprim/sulfamethoxazol(39.3%), 4 to ampicillin/sulbactam

(12.1%), 2 each to amoxicillin/clavulanic acid, nalidixic acid (6.0%), 1 each to tobramycin, gentamicin(3.0%). On the other hand, all of the strains were susceptible to cephalothin, colistin, kanamycin, amikacin, ceftriaxone, ceftazidime, ciprofloxacin, cefoxitin, cefotaxime.

Most of their resistance patterns appeared to be stable and there was no evidence of increased resistance throughout the 5 year period between 1998 and 2002.

The relative antimicrobial susceptibility of *S. flexneri* may vary geographically .[1,2,6] For examples, resistance to ampicillin, trimethoprim/sulfamethoxazol, chloramphenicol and nalidixic acid, agents typically used to treat shigellosis varies among geographic locations and then their continual monitoring is mandatory.

Notwithstanding the small number of isolates examined, the results of the present study showed similar patterns of antimicrobial resistance published by KNIH annual report. The present situation of high levels of resistance to multiple antibiotics has arisen principally because of the excessive and inappropriate use of these drugs in this region.

Table 1. Antimicrobial resistance phenotype and serotypes of 33 *S. flexneri* isolated in Busan

Resistance phenotype		Serotype(No. of strains)
Ⅰ:	Susceptible to all antimicrobials tested	2b(1)
Ⅱ:	Cb S C Te Am Tic	2a(16)
Ⅲ:	Cb S Te Am SAM Tic	variant Y(1)
Ⅳ:	SXT Cb S C Te Am Tic	2a(7), 5a(1)
Ⅴ:	Cb S C Te Am SAM Tic	2a(2)
Ⅵ:	Na SXT Cb S C Te Am Tic	3a(1)
Ⅶ:	SXT Cb S C AmC Te Am Tic	2a(2)
Ⅷ:	NN Gm SXT Cb S C Te Am Tic	2a(1)
Ⅸ:	Na SXT Cb S C Te Am SAM Tic	3a(1)

Abbreviations : Na, Nalidixic acid; NN, Tobramycin; Gm, Gentamicin; SXT, Trimethoprim/Sulfamethoxazol; Cb, Carbenicillin; S, Streptomycin; C, Chloram-phenicol; AmC, Amoxicillin/Clavulanic acid; Te, Tetracycline; Am, Ampicillin; SAM, Ampicillin/Sulbactam; Tic, Ticarcillin

Resistance should be recorded locally, and empiric therapy for suspected shigellosis should be changed accordingly.

Plasmid profiles

Some representative profiles are illustrted in Fig. 1. The plasmids in each of the isolates are shown in the Table 2.

The 33 clinical isloates exhibited 11 distinct profile patterns, although plasmid of the same size were present in multiple strains; for example, c. 1.7kb, 2.1kb and 18kb band were identified in most of 31 strains, c. 2.7kb band in three strains, c. 3.0kb

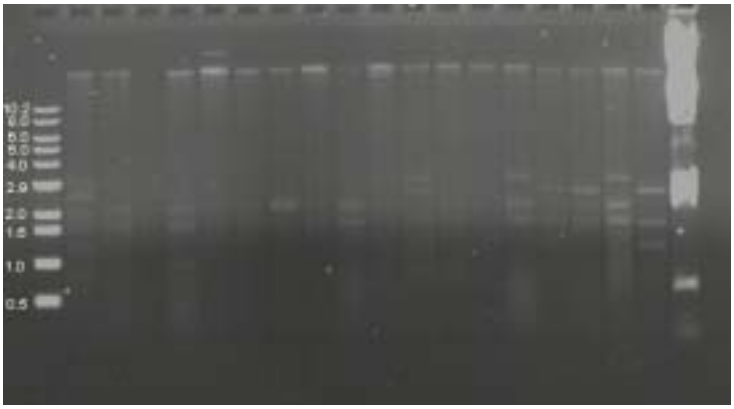

Fig. 1. Agarose gel electrophoresis of plasmid from some representative *S. flexneri* strain.

Table 2. Plasmid profiles and serotype of 33 *S. flexneri* isolated in Busan

Plasmid profiles(kb)	Resistance phenotype (No. of strains)	Serotype (No. of strains)
ND	Ⅱ(2)	2a(2)
18, 3.0, 2.7, 2.1, 1.7, 1.2, 0.8	Ⅵ(1)	3a(1)
18, 2.1, 1.7	Ⅱ(11), Ⅲ(1), Ⅳ(3), Ⅴ(2)	2a(16), varent Y(1)
18, 2.1, 1.7, 1.0	Ⅷ(1)	2a(1)
23, 18, 2.1, 1.7	Ⅳ(2)	2a(2)
18, 4.0, 2.1, 1.7	Ⅰ(1)	2b(1)
18, 17, 3.0, 2.1, 1.7	Ⅳ(1)	2a(1)
18, 3.0, 2.7, 1.7	Ⅱ(1), Ⅸ(1)	2a(1), 3a(1)
18, 3.0, 2.1, 1.7	Ⅳ(2), Ⅶ(2)	2a(3), 5a(1)
18, 2.7, 2.1, 1.7	Ⅱ(2)	2a(2)
18, 2.7, 1.7, 1.2	*Shigella flexneri* ATCC9403	3a

Abbreviations : ND, none detected

band in four strains.

The type strain *S. flexneri* ATCC 9403 harboured four plasmids with two same size of isolates(18kb, 1.7kb).

The maximum number of plasmids in any one isolate was seven and the mininum was none(two strains) with plasmid size ranging from c. 0.8kb to c. 23kb. However, there was no correlation between serotype and plasmid DNA profiles because one serotype had variable profile. (e.g. 2a serotype had 8 different plasmid profile)

And we could not find any specific correlation between serotype and antimicrobial resistance because one serotype had variable phenotype. (e.g. 2a serotype had 5 different phenotype)

The determination of plasmid profiles can aid in the differentiation of isolates and has been shown to be a useful tool in investigating the epidemic.

In this present study, plasmids of certain size(e.g. 1.7kb, 2.1kb and 18kb) were detected at higher frequencies and this characteristic might allow them to be used in the investigation of outbreaks caused by *S. flexneri* in this region.

국 문 초 록

1998년부터 2002년 부산지역에서 분리된 *Shigella flexineri* 33주에 대한 역학적 추적자로서의 antibiogram, serotype, plasmid profile을 상호 비교하였다.

분리균주의 혈청형별 분포는 2a가 28주로 가장 많았고 다음으로 3a가 2주, 2b, 5a, variant Y가 각각 1주씩으로 나타났다.

항균제 감수성 시험 결과는 전 약제에 감수성을 보이는 1주를 제외한 32주가 6제 이상의 다제내성을 보였으며 carbenicillin, streptomycin, chloramphenol, tetracycline, ampicillin, ticarcillin에는 100% 내성을 보임으로 기존의 싼 치료제는 더 이상 치료효과가 없음을 알수 있었다. 또한 치료제로서의 항균제의 선택시 원인균의 항균제 감수성시험이 전제되어야함을 알 수 있었다.

분리균의 Plasmid 보유현황은는 2주를 제외한 31주 모두 3개 이상 보유하였으며, 11개의 보유패턴으로 구분되었다. 그러나 분리균의 분자역학적 추적자로서의 antibiogram, serotype, plasmid profile을 조사해본 결과 특별한 상호관련성은 찾을 수 없었다.

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