Table 3. Retrospective analysis on the Incidence of *Vibrio fluvialis* among patients with acute diarrhea

Year	No of stool specimens screened	No. of *Vibrio cholerae* identified as non-O1, non-O139 (%)	No. of isolates identified as *Vibrio fluvialis* (%)	% incidence of *Vibrio fluvialis*
2000	2028	30	15 (50)	0.74
2001	2131	49	21 (43)	0.98
2002	2285	75	16 (21.3)	0.70
2003	1673	64	8 (12.5)	0.48
2004	2430	52	19 (36.5)	0.78
2005	1472	59	17 (29)	1.15
2006	930	33	12 (36.4)	1.3

Table 4. Detection of virulence genes and other marker genes among *V. parahaemolyticus* isolated from acute diarrheal patients in Kolkata

O:K Serovar(nos) n=34	Virulence and other marker gene profile (%)
3:K6 (5), 1:25 (5), UT:25 (3), UT:3 (2), 1:UT (2), UT:6 (2), 3:UT (2), UT:21, 4:8, UT:8	*tdh, toxRS* new, HUα (70.6)
3:6, 1:25, 2:4, 4:8, 4:63, UT:UT	*tdh, toxRS* new (17.6)
1:UT, 8:UT, 4:37, UT:3	*tdh* HUα (11.8)

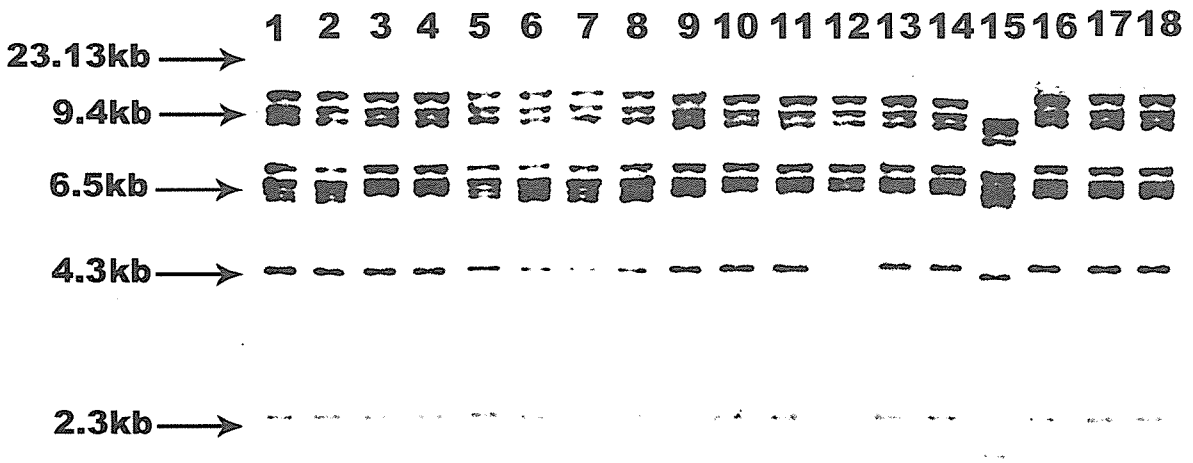

Fig 1. The *Bgl*I ribotype patterns of representative *V. cholerae* O1, Ogawa and Inaba isolates of the year 2004-2005 from Kolkata, India. Included isolates were O1 Inaba (Lanes 1-9) and Ogawa (lanes 10-18). Lanes 12 and 15 were new ribotypes. The positions of λ-*Hind*III markers run on the same gel are indicated on the left.

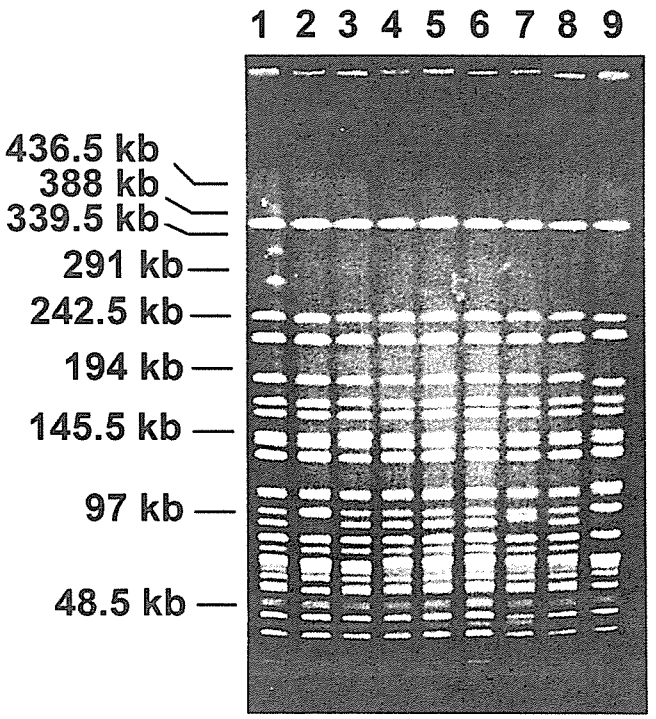

Fig. 2. PFGE profiles generated with *Not*I- digested genomic DNA obtained from representative *V. cholerae* O1, Inaba strains. Included *V. cholerae* O1 strains were as follows - Lanes: 1, CE 87; 2, J16173; 3, J22467; 4, J28466; 5, K4074; 6, K8646; 7, K12918; 8, K17466; 9, K23868. The positions of PFGE λ-ladder are indicated on the left.

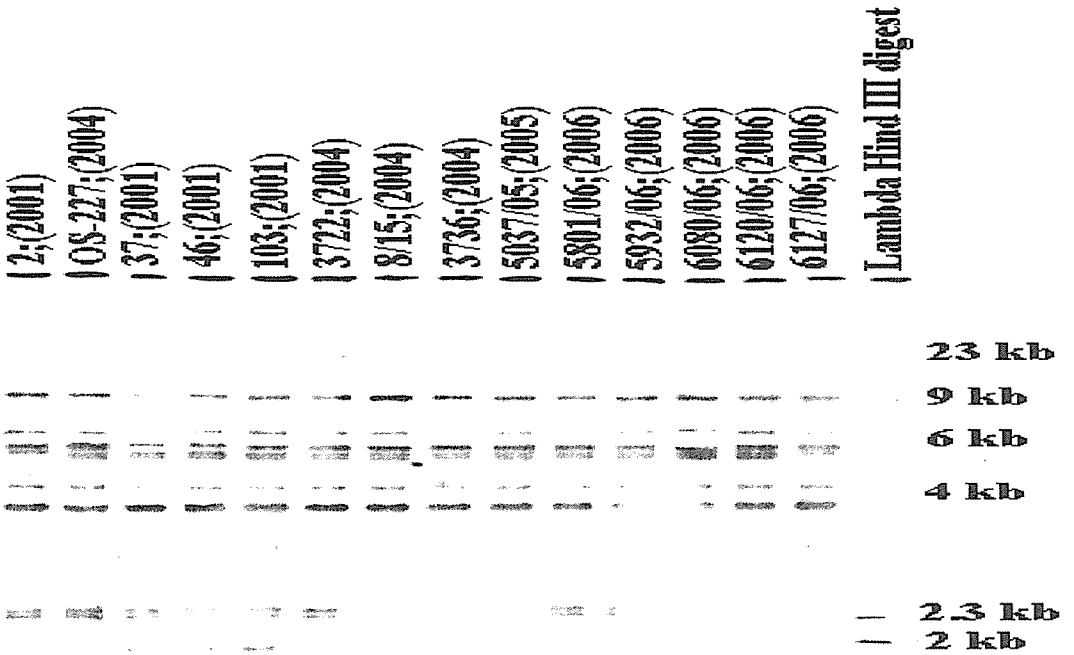

Fig. 3. The *BglI* ribotype patterns of representative *V. cholerae* O139 isolates of the year 2001-2006 from Delhi, India. The positions of λ-*Hind*III markers run on the same gel are indicated on the left.

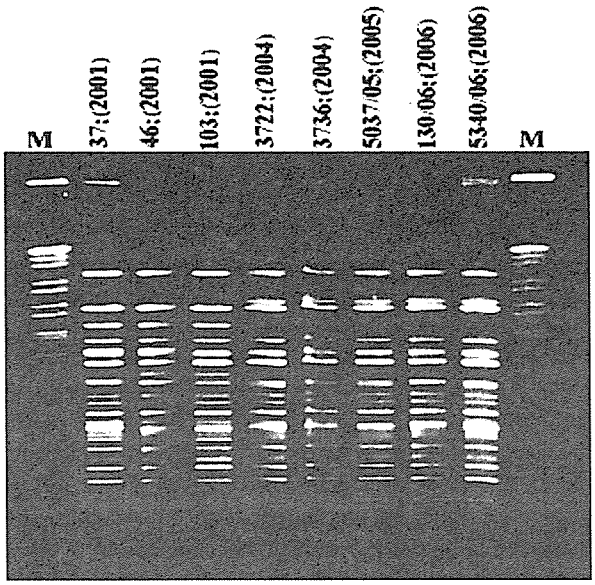

Fig. 4a

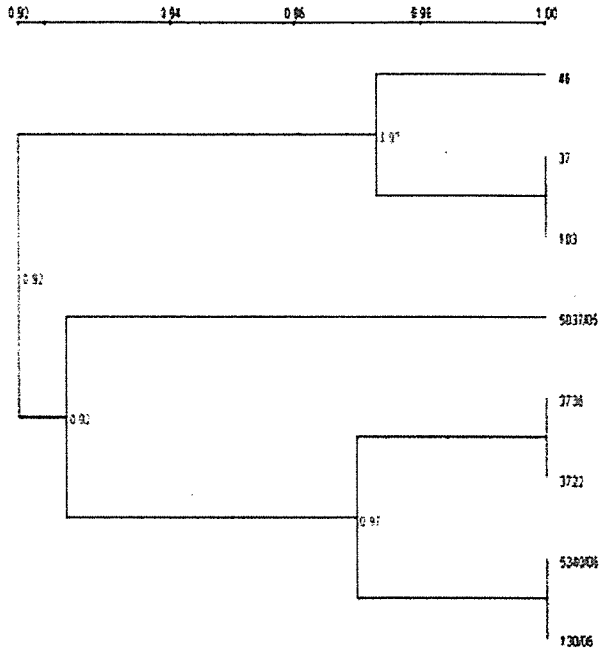

Fig. 4b

PFGE profiles generated with *Not*I- digested genomic DNA obtained from representative *V. cholerae* O139 isolates from Delhi. *Salmonella* serovar Braenderup H9812 strain was used as marker after digestion with *Xba*I (Fig 4a). Dendrogram using Diversity Data base showed two clusters (Fig. 4b) one with 2001 and the other with 2004 and 2006 isolates.

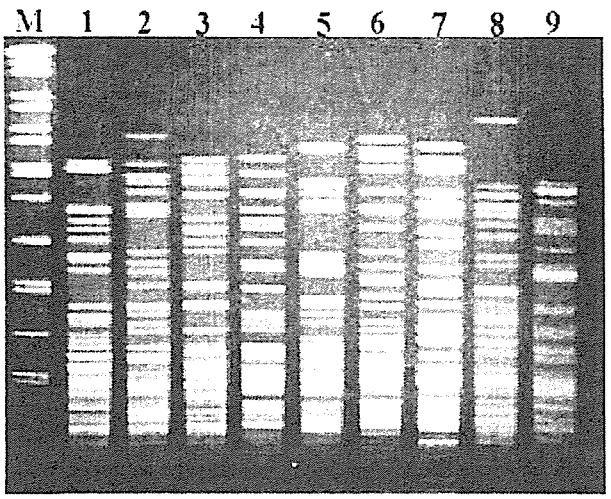

Fig. 5. PFGE profiles generated with *Not*I- digested genomic DNA obtained from representative *V. fluvialis* isolates from Kolkata. *Salmonella* serovar Braenderup H9812 strain was used as marker after digestion with *Xba*I.

Title: Virulence Factors and Molecular Epidemiology of Bacteria Causing Food-borne Poisoning Isolated in Thailand

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Summary: 300~500 words:

Food-borne pathogenic bacteria including *Vibrio parahaemolyticus,* Shiga toxin-producing *E. coli* (STEC), Enterotoxigenic *E. coli* (ETEC), and *Staphylococcus aureus* isolated from diarrhea patients or food samples during year 2005-2006 were characterized for their virulence factors by mean of phenotypic and genotypic.

A total of 542 *V. parahaemolyticus* isolates were agglutinated with commercial available 11 O and 71 K antiserum, determined for the presence of virulent hemolysin genes (*tdh* and *trh*) by a duplex PCR, and tested for urease production. The most frequent serotypes of *V. parahaemolyticus* were O3:K6 (28.4%), O4:K9 (9.6%), O4:K68 (8.5%), O4:K55 (7.2%), and O1:KUT (6.3%), respectively. During year 2005-2006, four outbreaks of *V. parahaemolyticus* caused by serotypes O3:K6, O4:K9, O4:K55 and O4:K68 occurred in different places. About 90% of *V. parahaemolyticus* strains including all outbreaks strains were *tdh* genes positive but *trh* genes and urease production negative. The outbreak strains of serotypes O3:K6, O4:K9, O4:K68 and O4:K55 were analyzed for molecular typing by PFGE of *Not*I digested genomic DNA. PFGE patterns of outbreak strains of the same serotypes showed minor different PFGE subtypes with more than 90% similarity. PFGE pattern of outbreak strains of serotype O4:K68 showed 75% similarity to PFGE pattern of outbreak strains of serotype O3:K6, suggesting a clonal relationship between the two serotypes.

A total of 3,638 *E. coli* isolates obtained from diarrheic patients were identified as ETEC and STEC by determination of heat-labile (*eltIA*) and heat-stable (*stIA*) enterotoxin genes of ETEC and shiga toxin genes (*stx1/2*) of STEC by a multiplex PCR. In addition, all isolates were determined for their O-serogroups by agglutinating with commercial available 43 O-antisera. Of the 3,638 *E. coli* isolates, 1.6% and 0.3% were ide[illegible]s

ETEC and STEC, respectively. Heat-labile or heat-stable enterotoxins genes positive were found in 45.6% each of ETEC. Only 8.8% of ETEC were both heat-labile and heat-stable enterotoxins genes positive. About 58% of ETEC were distributed in 11 O-serogroups and 42% were O-untypable. STEC strains found in this study were O157: non-motile (NM) and O111: NM.

A total of 92 *S. aureus* isolates obtained from patients in year 2005-2006 were characterized for antimicrobial susceptibility, bacteriophage type, and the presence of enterotoxins genes (*sea, seb, sec, sed,* and *see*). Methicillin-resistance (MRSA) and Methicillin-susceptible (MSSA) were found in 1 isolates (1.1%) and 91 isolates (98.9%) of *S. aureus*, respectively. MRSA and MSSA strains were susceptible to vancomycin, teicoplanin and fosfomycin. Mixed group of phages was found predominantly in 63.0% of *S. aureus.* About 32.6% of *S. aureus* harbored enterotoxins genes. A total of 130 *S. aureus* isolates obtained from patients in year 2004-2006 were analyzed for molecular typing by PFGE of *Sma*I digested genomic DNA.

This study demonstrated the emergence of *V. parahaemolyticus* serotypes O4:K68, in addition to O3:K6, as the possible new pandemic clone in Thailand. The PFGE patterns of outbreak strain of serotypes O4:K68 and O3:K6 showed clonal relationship. Moreover, this study also demonstrates the emergence of pathogenic STEC O157: NM.

Purpose:

Food-poisoning is still one of the major health problems in Thailand. In year 2003, 131,561 cases of food-poisoning with infection rate of 209.04 per 100,000 populations were reported to Epidemiology Division, Department of Diseases Control, Ministry of Public Heath, Thailand[(1)]. During the past ten years (1994-2004) the infection rate of food poisoning becomes increasing from 113.61/100,000 populations in year 1994 to 209.04/100,000 populations in year 2004. Though, the exact number of causative agents of food-poisoning is not available, many outbreaks of food poisoning were caused by *Staphylococcus aureus* and *Vibrio parahaemolyticus*. *S. aureus* is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents. Since 1997, infections cause by Methicillin-resistance *S. aureus* (MRSA) with intermediate susceptibility to

vancomycin (VISA)(MIC 8-16 μg/L) have been reported from Japan, France, the United states, Korea, Germany and Thailand. *V. parahaemolyticus* is one of the most important food-borne pathogen in Thailand, causing approximately half of the food-poisoning outbreaks. Since first observed in India in 1996, the pandemic O3:K6 clone of *V. parahaemolyticus* have been reported to cause outbreaks in many regions such as India, Russia, Southeast Asia, Japan and North America [2, 3] . In addition, Shiga toxin-producing *E. coli* (STEC) O157:H7 caused large outbreaks of food poisoning in many countries. Recently, food-poisoning due to STEC non-O157:H7 such as O26, O111, and etc, have been reported in many countries. Enterotoxigenic *E. coli* (ETEC) cause infantile diarrhea in many developing countries and traveler's diarrhea in developed countries. Recently, an attention of food-borne pathogens are raised that may due to the emergence changes of non-pathogenic to more severe pathogenic strain. In addition, the rapid transportation between country and country support the spread of an emergence pathogen through out the world. Therefore, well characteristic of pathogens are needed for prevention and control of an outbreak of an emergence food-borne pathogen. In Thailand, characteristic of food-borne pathogens is limited and not enough for making warning sign to public. Therefore, this study was designed to cover epidemiology and virulence characteristics of *V. parahaemolyticus*, STEC, ETEC, and *S. aureus*, obtained from diarrheic patients or food samples.

Methods:

1.1. Bacterial strains

Rectal swabs (one per patient) were collected from patients with acute diarrhea who attended hospitals across Thailand. Rectal swab samples collected in Cary-Blair transport medium were inoculated directly onto selective media including Sorbitol Mac-Conkey agar (SMAC), Mac-Conkey agar, Thiosulfate-Citrate-Bile salt-Sucrose (TCBS) agar, *Salmonella-Shigella* agar, Xylose Lysine Desoxycholate agar, Mannital salt agar, Selenite broth, and Alkaline peptone water for culture overnight at 37°C. In addition, food sample or swabs from food samples were cultured as the same manner. Sorbitol non-fermenting colonies were presumptively screened for STEC O157 by agglutinating with *E. coli* O157 antiserum. One to three lactose-fermenting and any lactose

non-fermenting colonies with typical *E. coli* morphology were initially selected. *S. aureus, V. parahaemolyticus,* and *E. coli* isolates, which had been presumptively identified at the hospitals concerned, were submitted to Enteric-Bacteria Laboratory, National Institute of Health, Thailand, for confirmation and further investigation.

V. parahaemolyticus and *E. coli* isolates were confirmed by standard methods[4,5]. In addition, *V. parahaemolyticus* isolates were tested with Urea agar supplemented with NaCl at a final concentration of 1% (Christensen's method) for detection of urease production [6,7]. *S. aureus* isolates were identified by gram staining, coagulase production, latex agglutination test for identification of *S. aureus* (Pastorex Staph-Plus, BioRad) [23].

1.2 Anti-microbial susceptibility test

S. aureus were screened for Methicillin-resistance (MRSA) by using latex agglutination kit (MRSA screen kit, Denka Seiken, Japan) and then tested for antimicrobial susceptible with 14 kinds of antimicrobial disk including amoxicillin/clavulinic acid (AMC), chloramphenicol(CHL), clindamycin(CLI), cefoxitin (CEF), co-trimoxazole (SXT), erythromycin (ERY), fosfomycin (FOS), gentamycin (GEN), ofloxacin (OFL), oxacillin (OXA), penicillin G (PEN), tetracycline (TET), teicoplanin (TEI) and vancomycin (VAN) by disk diffusion agar method as described by CLSI [24].

1.3 Bacteriophage typing

S. aureus were examined for bacteriophage typing by heat-shock technique (heating a culture at 55°C for 3 min immediately before phage typing) [25]., using the international phage typing set issued by PHLS Central Public Health Laboratory, Colindale, UK [8,9] . Susceptibility to phages was determined by standard routine test dilution (RTD) at 1,000 x RTD. The phage typing set consisted of Lytic group I : 29, 52, 52A, 79, 80 ; Lytic group II : 3A, 3C, 55, 71; Lytic group III: 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85 ; Lytic group V : 94, 96 and Miscellaneous group : 81, 95.

1.4 Serotyping

V. parahaemolyticus and *E. coli* were determined for O and K or H typing with antiserum kit product of Denka Seiken, Japan, as described by the manufacturer.

1.5 Determination of virulence-associated genes by PCR

1.5.1 *V. parahaemolyticus* were examined for thermostable direct hemolysin (*tdh*) and TDH-related hemolysin (*trh*) genes by a duplex PCR [12].

1.5.2 *E. coli* were examined for the presence of enterotoxins genes (*eltIA, stIA*) of ETEC and Shiga toxin genes (*stx1/2*) of STEC by a multiplex PCR [13].

1.5.3 *S. aureus* were determined for enterotoxin genes (*sea*, *seb*, *sec*, *sed*, and *see*) by a multiplex PCR [10].

1.6 Pulsed-field gel electrophoresis

V. parahaemolyticus outbreak strains and Shiga toxin-producing *E. coli* strains were determined for their DNA fingerprints by pulsed-field gel electrophoresis (PFGE) of *Not*I and *Xba*I digested genomic DNA, respectively. PFGE of *Not*I digested DNA was performed by modification of the methods described by Chowdhury, N. R. *et al,* 2000[20] and Yeung, P. S. M. *et al,* 2002[21]. *V. parahaemolyticus* PFGE profile was performed with initial time of 2 second, final time of 40 second and run time 19 hours. PFGE of Shiga toxin-producing *E. coli* was performed by using the one day (24-28 h) standardized protocol for pulsed-field gel electrophoresis of *E. coli* O157:H7. *S. aureus* strains were determined for their DNA fingerprints by pulsed-field gel electrophoresis (PFGE) of *Sma*I digested genomic DNA. *S. aureus* PFGE profile was performed with initial time of 5 second, final time of 40 second and run time 21 hours.

PFGEs were run on CHEF-DR III and then PFGE patterns were analyzed by Syngene Gene Directory Application version 1.02.0.

Results:

V. parahaemolyticus

A total of 542 isolates of *V. parahaemolyticus* isolated from gastroenteritis patients during year 2005-2006 were confirmed and classified into 10 serogroups and 36 serotypes. The O groups 3 and 4 were found the first and the second most frequent which were accounted for more than 70% of the strains as shown in Table 1. The most predominant serotypes were O3:K6 (28.4%), O4:K9 (9.6%), O4:K68 (8.5%), O4:K55 (7.2%).and O1:KUT (6.3%), respectively. During this period, four outbreaks of *V.*

parahaemolyticus occurred in 4 different regions of Thailand. The first outbreak caused by O4:K68 that occurred in July 2005 at Roi Et province, located in the north-eastern of Thailand. The second outbreak occurred in October 2005 in Bangkok caused by O4:K55 . The third outbreak caused by O3:K6 in June 2006 at Nakhon Sawan province, located in the north of Thailand. The forth outbreaks happened in July 2006 at Samut Songkhram near Bangkok by O4:K9.

Of the 542 strains, 488 (90.0%) strains, including outbreaks strains of serotypes O3:K6 (33 strains), O4:K9 (35 strains), O4:K55 (31 strains), and O4:K68 (16 strains), were positive for *tdh* genes but negative for both *trh* genes and urease production, 11 strains (2.0%) were carried both genes and urease positive, 42 strains (7.8%) were negative for all, and 1 strains (0.2%) was only urease positive. Serotypes and virulence factors are shown in Table 2.

PFGE patterns of O4:K68, O4:K55, O3:K6 and O4:K9 were designated as A, B, C and D, respectively. PFGE patterns of outbreak strains of the same serotypes showed minor different PFGE subtypes with more than 90% similarity.

PFGE patterns of O4:K68 outbreak strains showed minor different, as designated as subtypes A1 and A2, pattern A1 was found in about 93% among outbreaks strains. PFGE patterns of outbreak and non-outbreak strains of O4:K68 from year 2004-2006 showed 2 different groups with 6 different patterns (Figure2), Group 1 consisted of patterns A1 and A2 which were closely related with 95% similarity; Pattern A1 was found the most in every year. Another group consisted of pattern A3-A6 that were related but found in less frequent than A1.

The PFGE of O4:K55 outbreak strains showed 3 little different patterns, B1 to B3, which were 90 % similarity (Figure1). Moreover, only pattern B1 was found in the strains isolated in the past 4 years since 2002.

Three PFGE patterns with 90% similarity were observed among outbreak strains of O3:K6, C1 to C3 (Figure1). Pattern C 1 was the major pattern. Pattern C1 and C2 were found in outbreak and non-outbreak strains. PFGE of outbreak and non-outbreak strains of O3:K6 strains during year 2002-2006 showed much genetic variation (Figure2).

PFGE of O4:K9 strains from outbreak showed 2 little different subtypes, D1 and D2 which are 90% similarity (Figure1). The pattern D 1 was found the most in every year.

PFGE pattern (A1 and A2) of outbreak strains of serotype O4:K68 showed 75% similarity to the PFGE pattern (C1, C2 and C3) of outbreak strains of serotype O3:K6, suggesting a clonal relationship between O4:K68 and O3:K6. (Figure1 and Figure2)

E. coli

A total of 3,638 *E. coli* isolates obtained from patients were submitted during year 2005-2006. All isolates were identified as ETEC and STEC by determination of heat-labile (*eltIA*) and heat-stable (*stIA*) enterotoxin genes of ETEC and shiga toxin genes (*stx1/2*) of STEC. Of the 3,638 *E. coli* isolates, 57 (1.6%) and 12 (0.3%) were identified as ETEC and STEC, respectively. Among 57 ETEC strains, 26(46.5%) were *eltIA* genes positive, 26 (46.5%) were *stIA* genes positive, and 5 (8.8%) were *eltIA* and *stIA* genes positive (Table 3). About 58% of ETEC were distributed in 11 O-serogroups and 42% were O-untypable as shown in Table 3. Ten strains of STEC isolated from a child with watery diarrhea were *stx*1/2 positive O157:H non-motile (NM) and 2 strains were *stx*1 positive O111:H NM as shown in Table 4. The STEC strains were further determined for the virulence genes (*eae*A and enterohaemolysin genes) of STEC O157:H7 by a duplex PCR. STEC O157:HNM were *eaeA* and enterohaemolysin genes positive indicating the high virulent of this strain that were similar to STEC O157:H7. STEC O111 strains were *eae*A positive but enterohaemolysin genes negative.

PFGE of *Xba*I digested DNA patterns of STEC strains O111:HNM found in year 2003, 2004, and 2006 were more than 90% similarity as shown in Figure 3. PFGE pattern of STEC O157:HNM was 75% similarity to PFGE pattern of STEC O157:H7 (EDL 931).

S. aureus

A total of 92 *S. aureus* isolates submitted in year 2005 (18 isolates) and 2006 (74 isolates) were screened for Methicillin-resistance (MRSA) and then tested for susceptible to 14 kinds of antimicrobial disk. Of the 92 *S. aureus* isolates, 1 (1.1%) and 91 (98.9%)

were identified as MRSA and MSSA, respectively. MRSA strains were resistance to almost all drugs, except fosfomycin, teicoplanin, and vancomycin. Of the 92 MSSA strains, 4 (4.3%) were susceptible to all drugs, 87 (94.6%) were susceptible to almost all drugs except penicillin G. Of the 92 *S. aureus*, 12 (13.0%) were phage-untypable, 9 (9.8%), 10(10.9%), 1(1.1%), 2(2.2%) and 58(63.0%) were phage type lytic group II, III, V, miscellaneous group, and mixed group, respectively (shown in Table 1). Thirty-one (33.7%) of the 92 *S. aureus* were enterotoxins genes positive. Among enterotoxin genes–positive strains, 13 strains were isolated from stools and 11 strains were isolated from foods. Enterotoxins A, B, C, D and E genes (*sea, seb, sec, sed, see*) were found in 1 (1.1%), 5 (5.4%), 2 (2.2%), 1 (1.1%), and 22 (23.9%) strains, respectively. (Table 2.)

A total of 130 *Staphylococcus aureus* isolates were analyzed by PFGE of *SmaI* digested chromosomal DNA. Approximately 12-15 fragments ranging from 50-650 kb were found. A similarity index was determined for each pair of strains by using the Dice coefficient, with 2.0% band tolerance. The dendrogram generated with standard clustering software, the unweighted pair group method with arithmetic means (UPGMA). PFGE pattern of the 130 *S. aureus* isolates showed 120 in different types. There are 9 strains were most closely related with 80-90 %similarity and 2 strains were related with 75 % similarity. A total of 119 isolates were epidemiologically unrelated. (Figure 4.). *S.aureus* strains produced enterotoxin B in year 2004 and 2006 showed different PFGE types (Figure 5.). Comparison of *S. aureus* strains isolated from different provinces of in year 2005 also showered different PFGE types (Figure 6.) ,while *S. aureus* strains isolated from outbreak in the same province in year 2006 showed in the same patterns (Figure.7)

Discussion:

V. parahaemolyticus are diverse serotypes, 75 different combinations of O and K serotypes are recognized. After year 1996, besides O3:K6, other serotypes including O4:K68, O4:K8, O4:K12, O4:KUT, O1:K25, O1:K41, O1:K56, O3:K75, O1:KUT, and O5:KUT have emerged and been shown to belong to the pandemic clone by molecular typing techniques [2]. In this study, among 37 serotypes of *V. parahaemolyticus* found, O3:K6, O4:K9, O4:K68, O4:K55 and O1:K[illegible] were found the most frequent, respectively.

In addition, four outbreaks of *V. parahaemolyticus* caused by O4:K68, O4:K55, O3:K6, and O4:K9 occurred during this study. The O4:K68 was firstly emerged in Thailand in year 2004 as sporadic cases and was increasingly found in the 2005 outbreaks. This study indicates the emergence of pandemic O3:K6 and the possibility of emergence of pandemic O4:K68 and O1:KUT serotypes in Thailand.

V. parahaemolyticus strains that produce thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH), which are encoded by *tdh* and *trh* genes, respectively, are considered pathogenic [6]. In this study more than 90% of *V. parahaemolyticus* strains were *tdh* genes positive. All strains of serotypes O3:K6, O4:K9, O4:K55 and O4:K68 were *tdh* genes positive indicating the presence and the emergence of pathogenic pandemic strains in Thailand. In common with other *Vibrio* species, generally only a small population of clinical *V. parahaemolyticus* strains produces urease.

The urease positive phenotype of *V. parahaemolyticus* strains has been shown to be associated with the possession of the *trh* gene, and that the association is due to a genetic linkage between the structural genes of urease (*ure*) and *trh* on the chromosome. The *tdh* gene encodes thermostable direct hemolysin (TDH) that is the major cause of gastroenteritis [14,22].In this study, 2.2 % of the 542 *V. parahaemolyticus* strains were urease positive and they belonged to serotypes O1:K56, O1:K69, O1: KUT, O5:K15, O6:K18 and O10:KUT. The prevalence of urease-positive *V. parahaemolyticus* strains in this study is lower than previous reports that 4% and 7.5 % of urease-positive *V. parahaemolyticus* were found in Taiwan during 1992 and 1995 [15] and in Thailand in 1995 [16] . In this study, the difference between urease-positive strains was observed in that one of the 12 urease-positive strains was *trh* negative, thus genetic variation between the urease-positive strains needs to be further investigated.

In this study, PFGE patterns of outbreak strains of the same serotypes showed minor different PFGE subtypes with more than 90% similarity. Interestingly, the PFGE pattern (A1 and A2) of outbreak strains of serotype O4:K68 showed 75 % similarity to the PFGE pattern (C1 to C3) of outbreak strains of serotype O3:K6. Recently O4:K68 and O1:KUT *V.parahaemolyticus* from Asia have been reported to be closely related to the new O3:K6 clone according to molecular methods; therefore, concern has arisen that these

serotypes may potentially cause outbreaks and pandemic spread[2]. In this study, the PFGE pattern of outbreak strains of serotype O4:K68 showed relatively degrees of similarity to the PFGE pattern of outbreak strains of serotype O3:K6, suggesting a clonal relationship between O4:K68 and O3:K6 and formed what is referred to as the "pandemic group".

The prevalence of ETEC (1.6%) in this study shows that the prevalence of ETEC becomes decreasing comparing to three previous studies from Thailand. In1985 and 1986 [16, 17], ETEC (6% and 7%) and STEC (0% and 0%), as determined by probe hybridization, were recovered from 393 children in 16 district hospitals [16] and 278 children in the Children's Hospital in Bangkok [17], respectively. Moreover; ETEC (3%), and STEC (0.04%), as determined by PCR, were isolated from 2,100 Thai children during year 1996-2000 [18]. By the way, the virulence of ETEC in Thailand do not change much since the heat-stable enterotoxin probes/genes-positive ETEC and the both heat-labile and heat-stable enterotoxins probes/genes-positive ETEC are still the major and the minor groups, respectively. Our previous study (unpublished data) showed that prior to the year 2003, STEC strains were very rare in Thailand. Since year 2003, STEC strains were gradually increasing in every year and STEC O111:HNM were found to become increasingly. Though STEC O111:HNM strains were less virulence than STEC O157:H7, large outbreaks of STEC O111 have been reported in many developed countries. Moreover, in this study we also found STEC O157:HNM that were as high virulence as STEC O157:H7. STEC O157:HNM also have been reported as the causative of outbreaks in developed country. Though, the isolation rate of STEC is still low, care and awareness of outbreak of STEC should be taken and laboratory surveillance of STEC needs to be continuing performed.

Among the *S. aureus* strains isolated from food samples the percentage of enterotoxicgenic strains is estimated to be around 25%[27]. In our study during year 2005 to 2006 , it was found that 32.6% of *S. aureus* strains produced enterotoxin. MSSA producing enterotoxin E is widely spread among the general population. Enterotoxin B, C, D and E were found in 5.4%, 2.2%, 1.1% and 23.9% , respectively, during year 2005-2006. The emergence of MRSA (1.5%) was found during year 2005, they were enterotoxins genes negative and susceptible to vancomycin. About 87.0% of *S. aureus* were phage typable and

distributions of phage types were unspecific in each year. PFGE is considered the gold standard technique for *S. aureus* typing due to it high discriminatory power, its excellent reproducibility, and its good correlation with epidemiologically linked data[28]. This method gave 120 different patterns due to most of *S. aureus* strains were isolated from sporadic cases. *S. aureus* strains that had the same enterotoxin and phage type also gave different PFGE patterns. PFGE is more appropriate and provides more information due to its high discriminatory power.

In conclusion this study demonstrates the emergence of *V. parahaemolyticus* serotypes O4:K68, in addition to O3:K6, as the possible new pandemic clone in Thailand, and also demonstrates the emergence of pathogenic STEC O157:NM This study provides an information to an early warning and may contribute to a preparedness for prevention and control of possible outbreaks of emerging of new pandemic cone of *V. parahaemolyticus* and STEC O157.

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