Antimicrobial Resistance Surveillance of *Vibrio cholerae* in Thailand from 2000 to 2004

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Objective: *To study the trends of antimicrobial resistance pattern of Vibrio cholerae in Thailand between 2000 and 2004.*
Material and Method: *All isolates of Vibrio cholerae from 28 hospitals across Thailand between 2000 and 2004 were tested for their susceptibility to ampicillin, chloramphenicol, norfloxacin, tetracycline and trimethoprim/sulfamethoxazole by the disk diffusion method (Kirby Bauer). The relevant data were collected and analyzed by the WHONET software program supported by the World Health Organization (WHO).*
Results: *V. cholerae O1, serotype Inaba was much more common than serotype Ogawa. The most frequent type of clinical specimens that V. cholerae isolated was the stool. There was no trend of increasing resistance of all V. cholerae both O1 and non O1. Over all average rates of tetracycline resistance of V. cholerae O1, Inaba and Ogawa were 0.9% and 16.3% respectively and trimethoprim/sulfamethoxazole resistance were 0.4% and 60.5% respectively. The strains were not resistant to norfloxacin.*
Conclusion: *In Thailand, V. cholerae O1 were still susceptible to tetracycline and norfloxacin which were the most frequently antimicrobial used for the treatment of cholera. The trend of increasing resistance during the study period was not detected.*

Keywords: *Anti-infective agents, Drug resistance, bacterial, Microbial sensitivity tests, Population surveillance, Thailand, Vibrio cholerae*

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The National Antimicrobial Resistance Surveillance Center of Thailand (NARST) was established in 1998 in the National Institute of Health (Thai NIH) of the Department of Medical Sciences with support of the World Health Organization (WHO). The center was designated as the WHO Collaboration Center on Antimicrobial Resistance Surveillance and Training in 2005. It has been the coordinator of the surveillance network using WHONET software. Work of the NARST has been supported by the Thai NIH, the national reference laboratory that supports antimicrobial susceptibility testing performed by the network's laboratories. The NARST also provides training to the network on use of the software, connecting, integrating and interpreting the surveillance data.

The NARST in collaboration with network hospitals has collected data on antimicrobial resistance of many bacteria including cholera. *Vibrio cholerae* is one of the most common causes of infectious diarrhea worldwide. Its manifestation ranges from mild to fatal, profound watery diarrhea. It can cause endemic and epidemic outbreaks. Antibiotic therapy reduces the duration of symptoms and bacterial shedding[1]. The most commonly used antimicrobial agents are tetracyclines and quinolones[2-4]. Because both of

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them are widely used in Thailand for the treatment of infections in the community in humans and animals, therefore, their inappropriate use may result in an increase in resistance in the community. The resistance may spread to *V. cholerae* in the community and may result in a failure to treatment with those antimicrobial agents. Therefore, the present study aimed to explore the resistance trends of *V. cholerae* to the commonly used antibiotics for treatment of infective diarrhea in Thailand and to increase awareness among medical professionals of the antibiotic resistance problem.

Material and Method

The NARST collected data on resistance of *V. cholerae* to the commonly used antibiotics from totally 28 hospitals participating in the surveillance network, including 19 large hospitals (more than 500 beds) and 9 small hospitals (equal or less than 500 beds). The data collected routinely by clinical laboratories in the hospitals using WHONET have been sent to the Thai NIH on a quarterly basis. The data collected between 2000 and 2004 were on a number of antibiotics, *i.e.* ampicillin, chloramphenicol, norfloxacin, tetracycline and trimethoprim-sulfamethoxazole. The data were analyzed using the WHONET software program.

Microbiology

Isolation and identification of *V. cholerae* were performed following standard bacteriological method[5,6] and antimicrobial susceptibility tests were performed by using the Kirby Bauer disk diffusion method following the method of Clinical and Laboratory Standards Institute (CLSI)[formerly National Committee for Clinical Laboratory Standards (NCCLS)][7,8].

Result

Isolates

A total of 1,269 *V. cholerae* strains isolated from patients from 2000 to 2004 were obtained; 1,234 and 35 strains were obtained from stool and blood, respectively. The majority of strains isolated from stool were *V. cholerae* O1; 1,148 (93%) isolates were Inaba serotype, and only 45 (3.7%) were Ogawa serotype, and 41 were *V. cholerae* O139 (3.3%). Thirty-five strains of *V. cholerae* non O1/non O139 were isolated from blood (Table 1).

Antimicrobial resistance

V. cholerae O1, serotypes Inaba and Ogawa

During the five-year period of study between 2000 and 2004, ampicillin, chloramphenicol, norfloxacin, tetracycline and trimethoprim-sulfamethoxazole resistance rates of *V. cholerae* O1, serotype Inaba ranged from 0% to 7.2%, 0% to 0.7%, 0% to 0.2%, 0 % to 1.3%, and 0% to 0.9%, respectively. The rates of antimicrobial resistance had not increased over the study period (Table 2).

The average rates of antimicrobial agent resistance among *V. cholerae* O1, serotype Ogawa from 2000 to 2004 are as follows: 60.5% was resistant to trimethoprim/sulfamethoxazole; 16.3% was to tetracycline; 7.3% was resistant chloramphenicol; and 4.4% was resistant to ampicillin. All isolates were not resistant to norfloxacin (Table 3).

V. cholerae non O1/non O139

During the study period, 20% (35 cases) of *V. cholerae* non-O1/non-O139 isolated from the blood were resistant to trimethoprim-sulfamethoxazole; 13.6% was resistant to tetracycline; and 10% was resistant to ampicillin. All isolates were not resistant to chloramphenicol and norfloxacin.

Discussion

The five-year resistance surveillance from 2000 to 2004 in Thailand revealed Inaba as the main serotype, followed by *V. cholerae* O1, serotype Ogawa. *V. cholerae* O139 was least resistant to all antimicrobial tested. The antimicrobial resistance rate of *V. cholerae* O1, serotype Inaba was very low, whereas the rate of resistance of serotype Ogawa was higher than that of Inaba. In addition, *V. cholerae* non-O1/non-O139 isolates was less resistant than *V. cholerae* O1 both Inaba and Ogawa.

The resistance to antimicrobial agents of *V. cholerae* O1 sent from all over the country for

Table 1. *Vibrio cholerae* O1, Inaba, Ogawa and *V. cholerae* O139 strain isolated from the stool and *V. cholerae* non O1/non O139 from blood from year 2000 to 2004 in Thailand

Vibrio cholerae	No. of strain					Total (No.)
	2000	2001	2002	2003	2004	
O1						
Inaba	106	457	225	185	175	1,148
Ogawa	32	3	5	5	0	45
O139	9	2	2	10	18	41
non O1/non O139	4	6	10	8	7	35
Total (No.)	151	468	342	208	200	1,269

Table. 2 Antimicrobial resistance of *Vibrio cholerae* O1, Inaba isolated from rectal swab and stool, from 2000 to 2004 in Thailand

Year	Ampicillin		Chloramphenicol		Norfloxacin		Tetracycline		Trimethoprim-Sulfamethoxazole	
	No. test	R (%)	No. test	R (%)	No. test	R (%)	No. test	R (%)	No. test	R (%)
2000	102	0	93	0	67	0	100	0	102	0
2001	445	7.2	418	0.7	431	0.2	452	1.3	403	0.2
2002	208	1.9	193	0	218	0	224	0.9	222	0.9
2003	170	1.2	133	0	177	0	179	0.6	179	0.6
2004	81	3.7	54	0	92	0	77	0	98	0
Total	1,006	4.1	891	0.3	985	0.1	1,032	0.9	1,004	0.4

Table. 3 Antimicrobial resistance of *Vibrio cholerae* O1, Ogawa isolated from the rectal swab and the stool from 2000 to 2004 in Thailand

Year	Ampicillin		Chloramphenicol		Norfloxacin		Tetracycline		Trimethoprim-Sulfamethoxazole	
	No. test	R (%)	No. test	R (%)	No. test	R (%)	No. test	R (%)	No. test	R (%)
2000	32	6.3	32	9.4	32	0	31	12.9	32	78.1
2001	3	0	3	0	2	0	3	33.3	1	0
2002	5	0	3	0	3	0	5	40	5	20
2003	5	0	3	0	5	0	4	0	5	0
2004	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total	45	4.4	41	7.3	42	0	43	16.3	43	60.5

Table 4. Antimicrobial resistance of *Vibrio cholerae* non-O1/non-O139 isolated from the blood, from 2000 to 2004 in Thailand

Year	Ampicillin		Chloramphenicol		Norfloxacin		Tetracycline		Trimethoprim-Sulfamethoxazole	
	No. test	R (%)	No. test	R (%)	No. test	R (%)	No. test	R (%)	No. test	R (%)
2000	4	25	4	0	1	0	2	0	4	0
2001	5	0	4	0	ND	ND	5	0	6	16.7
2002	8	0	7	0	1	0	6	33.3	10	40
2003	8	12.5	6	0	1	0	4	0	8	12.5
2004	5	20	3	0	1	0	5	20	7	14.3
Total	30	10	24	0	4	0	22	13.6	35	20

confirmation test at the Thai NIH between 2005 and 2007 remained the same as in the previous five years. It was found that the resistance rate of serotype Ogawa was higher than that of Inaba. *V. cholerae* O1 serotype Ogawa were still resistant to trimethoprim/sulfametho-xazole at higher rate continuingly by resisting at 99.2%, 93.8% and 97.7%, respectively from 2005 to 2007. However, *V. cholerae* O1 serotype Ogawa has also increased resistance to tetracycline up to 96% but there was no resistance to ampicilin, chloramphenicol and norfloxacin in 2007. The antimicrobial resistance of Inaba was very low from 2000 to

2004, and from 2005 to 2006, the resistance rate was still lower than 5% to ampicillin, chloramphenicol and tetracycline. Except for 2007, the isolates were 100% (12 isolates) resistant to trimethoprim/sulfamethoxazole but there was no resistance to ampicilin, tetracycline and norfloxacin.

In conclusion, laboratory surveillance found that the *V. cholerae* O1, and serotype Ogawa often resisted to many antimicrobials especially to trimethoprim/sulfamethoxazole and tetracycline while serotype Inaba did not resist to any antimicrobials except the trimethoprim/sulfamethoxazole.

The primary treatment for patients with cholera is rehydration with oral or intravenous fluids. Antibiotics are given to decrease the volume of purging, the duration of diarrhoea, and thus to decrease the cost of treatment. Inexpensive, effective antibiotics are very cost-effective as adjunct therapy in severe cases, since they reduce the hospital stay and decrease the volume of intravenous fluids and oral rehydration solution (ORS) needed for rehydration. While antibiotics rapidly eradicate organisms from the stool, they probably have minimal impact on the dynamics of cholera transmission in the community, as there are environmental reservoirs and because a large proportion of asymptomatic, or only mildly ill, infected individuals, who would not normally receive antibiotics, shed vibrio[9].

Tetracycline has traditionally been the antibiotic of choice, but resistance to this drug is widespread. Trimethoprim/sulfamethoxazole is recommended as the first-line drug for children and furazolidone for pregnant women[10]. Resistance to Trimethoprim/sulfametho-xazole and tetracycline is increasing in Thailand between 2005 and 2007. However, the resistance pattern of *V. cholerae* O1 isolates seemed to fluctuate from year to year. It was suggested that if the clinicians need to use antimicrobial agents for treatment of cholera patients, they can use the first drug of choice as recommended by WHO such as tetracycline and doxycyline. Nevertheless, the anti-microbial agent resistance surveillance should be continuously maintained.

References

1. Greenough WB 3rd, Gordon RS Jr, Rosenberg IS, Davies BI, Benenson AS. Tetracycline in the treatment of cholera. Lancet 1964; 1: 355-7.
2. World Health Organization. Guidelines for cholera control. Geneva: WHO; 1993.
3. Yamamoto T, Nair GB, Albert MJ, Parodi CC, Takeda Y. Survey of in vitro susceptibilities of *Vibrio cholerae* O1 and O139 to antimicrobial agents. Antimicrob Agents Chemother 1995; 39: 241-4.
4. Bhattacharya SK, Bhattacharya MK, Dutta P, Dutta D, De SP, Sikdar SN, et al. Double-blind, randomized, controlled clinical trial of norfloxacin for cholera. Antimicrob Agents Chemother 1990; 34: 939-40.
5. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 18th Informational supplement M100-S18. Wayne, PA: CLSI; 2008.
6. World Health Organization. Program for control of diarrhoeal diseases. In Manual for laboratory investigation of acute enteric infection, CDD/83.3. Geneva: WHO; 1987.
7. Supawat K. *Vibrio cholerae* (classical) and *Vibrio cholerae* O139. In: Warachit P, Poonwan N, editors. Emerging infectious diseases: manual for laboratory testing. Bangkok: Department of Medical Sciences, Ministry of Public Health; 1998: 97-124.
8. Wikler MA, Low DE, Cockerill FR, Sheehan DJ, Craig WA, Tenover FC, et al. Performance standards for antimicrobial disk susceptibility tests; approved standard, 9th ed. Clinical and Laboratory Standards Institute document M2-A9. Wayne, PA: CLSI; 2006.
9. World Health Organization. Antimicrobial resistance in Shigellosis, Cholera and Campylo-bactoriosis. WHO/CDS/CSR/DRS/2001.8. Geneva: WHO; 2001.
10. World Health Organization. Antibiotic resistance synthesis of Recommendations by Expert Policy Groups. WHO/CDS/CSR/DRS/2001.10. Geneva: 2001.

การเฝ้าระวังการดื้อยาต้านจุลชีพของเชื้อวิบริโอ คลอเลอรี ในประเทศไทยระหว่างปี พ.ศ.2543-2547

กรองแก้ว ศุภวัฒน์, ศรีวรรณา หัทยานานนท์, ปฐม สวรรค์ปัญญาเลิศ, นลินี อัศวโภคี, ภิรุญ มุตสิกพันธ์

วัตถุประสงค์: เพื่อศึกษาแนวโน้มของการดื้อยาต้านจุลชีพของเชื้อวิบริโอ คลอเลอรีในประเทศไทย ระหว่างปี พ.ศ. 2543-2547

วัสดุและวิธีการ: ทดสอบความไวของเชื้อวิบริโอ คลอเลอรี ที่ได้มาจากโรงพยาบาลที่เข้าร่วมโครงการ 28 แห่ง ต่อยาต้านจุลชีพ แอมพิซิลิน, เตตร้าไซคลิน, ไตรเมโธพริม-ซัลฟาเมธ็อกซาโซล และ นอร์ฟลอกซาซิน ด้วยวิธีการทดสอบแบบ disk diffusion method (Kirby Bauer) วิเคราะห์ข้อมูลที่ได้ด้วยโปรแกรมคอมพิวเตอร์ WHONET ที่ได้รับการสนับสนุน จากองค์การอนามัยโลก

ผลการศึกษา: สายพันธุ์ที่พบบ่อยที่สุดของวิบริโอ คลอเลอรี คือ V. cholerae O1 (ร้อยละ 52) โดย พบ serotype Inaba มากกว่า Ogawa. สิ่งส่งตรวจทางคลินิกที่แยกเชื้อได้มากที่สุดคืออุจจาระ ยังไม่พบปัญหาแนวโน้มการดื้อยาสูงขึ้นของเชื้อวิบริโอ คลอเลอรีทั้ง O1 และ non O1 ดื้อยาปฏิชีวนะมากกว่า O1 โดยรวมอัตราเฉลี่ย การดื้อยาเตตร้าไซคลินของวิบริโอ คลอเลอรี O1 ซีโรทัยป์ Inaba,และ Ogawa เท่ากับร้อยละ 0.9 และ 16.3 ตามลำดับ และดื้อยาไตรเมโธพริม-ซัลฟาเมธ็อกซาโซลเท่ากับร้อยละ 0.4 และ 60.5 ตามลำดับ เชื้อไม่ดื้อต่อยานอร์ฟลอกซาซิน

สรุป: วิบริโอ คลอเลอรีในประเทศไทยยังไวต่อยาเตตร้าไซคลินและนอร์ฟลอกซาซินซึ่งเป็นยาปฏิชีวนะที่ใช้กันบ่อยที่สุดในการรักษาโรคอหิวาตกโรค ยังไม่พบว่ามีแนวโน้มการดื้อยาเพิ่มขึ้นในช่วงเวลาที่ศึกษา