

Survey of antibiogram tests in cholera patients in the 2005 epidemic in Hamadan, Islamic Republic of Iran

F. Keramat,¹ S.H. Hashemi,¹ M. Mamani,¹ M. Ranjbar¹ and H. Erfan²

تَقْصِي اختبارات الحساسية للمضادات الحيوية لدى مرضى الكوليرا في وباء 2005 في همدان، بجمهورية إيران الإسلامية

فريبا كرامت، سيد حميد هاشمي، مژگان مماني، ميترا رنجبر، حسين عرفاني

الخلاصة: أجرى الباحثون دراسة مستعرضة تحليلية للتعرف على المجموعات المصلية والأنماط المصلية لضمات الكوليرا ومعدلات مقاومتها للمضادات الحيوية في وباء الكوليرا الذي ضرب همدان عام 2005. وقد شملت الدراسة 190 مريضاً لديهم زرع براز إيجابي لضمات الكوليرا من المجموعة المصلية O1، والنمط البيولوجي الطور، والنمط المصلي إنابا. ومن بين 60 حالة اختارها الباحثون عشوائياً لإجراء اختبارات الحساسية للمضادات الحيوية، لوحظ أن الحساسية للنورفلوكساسين تبلغ 97% والسيبروفلو كساسين 92% والكاناميسين 88% والأميكاسين 85% والتترايسيكلين 77% والدوكسي سيكلين 67%. وأن المقاومة للفورازوليدون تبلغ 100% ولتريميثوبريم – سلفاميثو كسازول 98% ولإريثروميسين 62%. وتدل مقارنة هذه النتائج مع نتائج وباء 1988 على ارتفاع يبعث على القلق في زيادة مقاومة ضمات الكوليرا للإريثروميسين والدوكسي سيكلين والسيبروفلو كساسين.

ABSTRACT An analytical cross-sectional study determined the serogroups and serotypes of *Vibrio cholerae*, and their antibiotic resistance rates, in the 2005 cholera epidemic in Hamadan. All 190 patients with positive stool cultures had *V. cholerae* serogroup O1, biotype El Tor and serotype Inaba positive. Of 60 cases selected randomly for antibiogram testing, sensitivity to norfloxacin, ciprofloxacin, kanamycin, amikacin, tetracycline and doxycycline was 97%, 92%, 88%, 85%, 77% and 67% respectively. Resistance to furazolidone, trimethoprim-sulfamethoxazole and erythromycin was 100%, 98% and 62% respectively. Comparison with the results of the 1998 epidemic suggests a worrying increase in the resistance of *V. cholerae* to erythromycin, doxycycline and ciprofloxacin.

Étude des antibiogrammes chez les patients atteints de choléra lors de l'épidémie de 2005 à Hamadan (République islamique d'Iran)

RÉSUMÉ Une étude transversale analytique a déterminé les sérogroupes et les sérotypes *Vibrio cholerae*, ainsi que leur taux de résistance aux antibiotiques, lors de l'épidémie de choléra de 2005 à Hamadan. On a identifié chez l'ensemble des 190 patients, dont les cultures de selles étaient positives, *V. cholerae* du séro groupe O1, biotype El Tor, sérotype Inaba. Sur 60 cas choisis au hasard pour faire l'objet d'un antibiogramme, la sensibilité à la norfloxacine, la ciprofloxacine, la kanamycine, l'amikacine, la tétracycline et la doxycycline était respectivement de 97 %, 92 %, 88 %, 85 %, 77 % et 67 %. La résistance à la furazolidone, au triméthoprime-sulfaméthoxazole et à l'érythromycine était respectivement de 100 %, 98 % et 62 %. La comparaison avec les résultats de l'épidémie de 1998 semble indiquer une augmentation inquiétante de la résistance de *V. cholerae* à l'érythromycine, la doxycycline et la ciprofloxacine.

¹Department of Infectious Diseases, Sina Hospital, Hamadan University of Medical Sciences, Hamadan, Islamic Republic of Iran (Correspondence to F. Keramat: faribakeramat@yahoo.com).

²Centre for Prevention and Control for Diseases, Hamadan, Islamic Republic of Iran.

Received: 16/01/06; accepted: 08/05/06

Introduction

Cholera is an acute diarrhoeal disease that can, in a matter of hours, result in profound, rapidly progressive dehydration and death [1]. Since 1817, 7 global pandemics have occurred. The current (7th) pandemic, the first due to the El Tor biotype, began in Indonesia in 1961 and spread throughout Asia as *Vibrio cholerae* El Tor, displacing the endemic classic strain in many areas [1–3]. In October 1992, a large-scale outbreak of clinical cholera occurred in south-east India. This strain spread rapidly up and down the coast of the Bay of Bengal, reaching Bangladesh in December 1992. There it caused more than 100 000 cases of cholera in the first 3 months of 1993. It subsequently spread across the Indian subcontinent and to neighbouring countries, affecting Pakistan, Nepal, western China, Thailand and Malaysia by the end of 1994.

The organism has since been designated *V. cholerae* O139 Bengal [1]. Currently, in most regions of south-east Asia, *V. cholerae* serogroup O1 remains dominant, whereas in other regions serogroup O139 periodically re-emerges [1,2]. *V. cholerae* serogroup O1 is most common cause of cholera epidemics. In endemic areas, the disease is more common in the summer and autumn months [1]. Two biotypes of *V. cholerae* O1—classic and El Tor—are distinguished. Each biotype is further subdivided into 2 sero-types, termed Inaba and Ogawa [1,2]. An epidemic of cholera in Iraq was anticipated for the year 1999 and in Baghdad city 874 cases of cholera were reported during the epidemic, mostly *V. cholerae* El-Tor O1, serotypes Ogawa and Inaba [4].

The hallmark of cholera is the production of watery diarrhoea with varying degrees of dehydration ranging from none to severe and life-threatening diarrhoea. The goal of therapy is to restore the fluid loss caused by diarrhoea and vomiting.

Antimicrobial agents play a secondary role in the treatment of cholera. When patients with severe dehydration are given antibiotics, the duration of diarrhoea is decreased and the volume of stool is reduced by nearly half [1,2]. The Islamic Republic of Iran is at risk of epidemics spreading from neighbouring countries. So far there have been 12 epidemics of cholera with the 1st epidemic in 1965 (Figure 1). The Center for Disease Control at the Ministry of Health and Medical Education reported 1133 cases of cholera with 12 deaths in the epidemic of 2005.

Due to increasing reports of antibiotic resistance in strains of *V. cholerae* O1 [1,2], our objectives were to determine the serogroups and serotypes of *V. cholerae*, and their sensitivity and resistance to antibiotics, in the epidemic of cholera in 2005 in Hamadan province and to compare the results with those from the epidemic of 1998.

Methods

This survey was carried out using an analytical cross-sectional method in the summer of 2005, following the start of the epidemic of cholera in Hamadan, western Islamic Republic of Iran.

A total of 190 isolates of *V. cholerae* were obtained from patients suspected of cholera who were referred to health centres or hospitals. The specimens were collected on sterile swabs, which were then placed in Cary–Blair transport medium. Alkaline peptone water was used for the enrichment of *V. cholerae*, which was then isolated on thiosulphate-citrate-bile salt-sucrose (TCBS) agar [5]. Biochemical identification and serotyping were performed by standard procedures [6].

Susceptibility to antimicrobial agents was examined by an agar disk diffusion

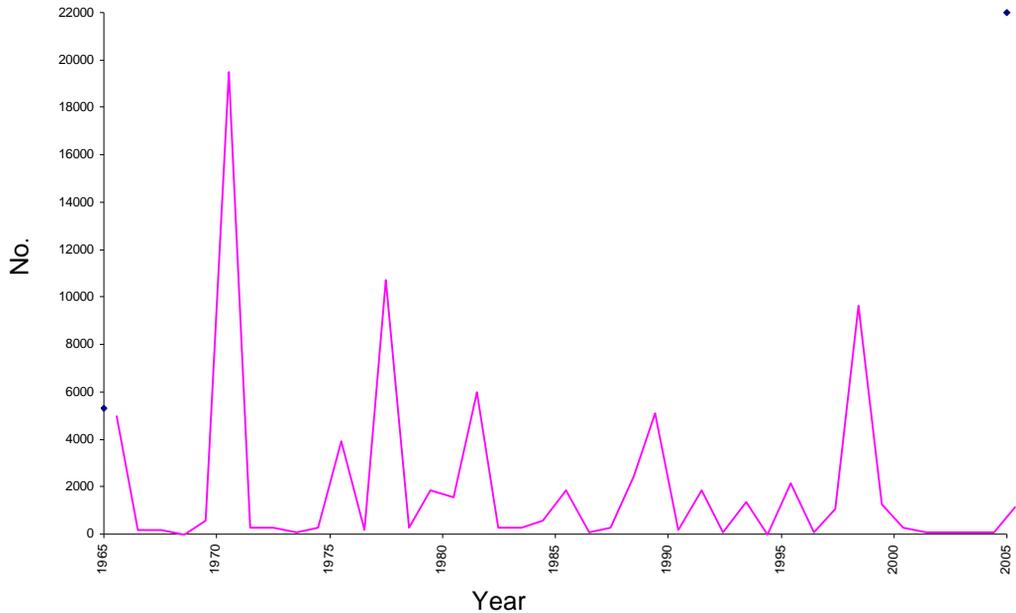


Figure 1 Trend of cholera cases in the Islamic Republic of Iran from 1965 to 2005 [Source: Center for Disease Control, Ministry of Health and Medical Education, Islamic Republic of Iran]

method on Mueller–Hinton agar [7]. The antibiotic disks were prepared by the Padtan Teb company (Tehran, Islamic Republic of Iran). The following antibiotics were used at the following concentration of drug per disk: amikacin (30 µg), tetracycline (30 µg), furazolidone (100 µg), norfloxacin (10 µg), erythromycin (15 µg), kanamycin (30 µg), doxycycline (30 µg), ciprofloxacin (5 µg), trimethoprim–sulfamethoxazole (25 µg). After 18 hours of incubation at 37 °C on TCBS, strains were characterized as susceptible or resistant based on inhibition zone sizes [5].

Results

Of the 13 172 specimens collected from patients suspected of infection with cholera, 190 (1.4%) were identified as *V. cholerae*

O1, biotype El Tor and serotype Inaba. Of the affected patients, 110 (58%) were males and 80 (42%) females, with 86% urban and 14% rural residence. A total of 8% of cases were admitted to hospital and the remainder were treated as outpatients. The incidence of *V. cholerae* in Hamadan province was 11 per 100 000 population. The case fatality rate was 1.1%.

From the total isolates, 60 specimens were selected randomly for further analysis: 33 (55%) from males and 27 (45%) from females. As Table 1 shows *V. cholerae* was most frequent in the age group 11–30 years (58%). The mean age of patients was 25.97 years.

The sensitivity of the *V. cholerae* strains to norfloxacin, ciprofloxacin, kanamycin, amikacin, tetracycline and doxycycline was 97%, 92%, 88%, 85%,

Table 1 Age distribution of patients with *Vibrio cholerae* (n = 60) in Hamadan, Islamic Republic of Iran in 2005

| Age (years) | No. | % |
|-------------|-----|----|
| 0–10 | 9 | 15 |
| 11–20 | 19 | 32 |
| 21–30 | 16 | 27 |
| 31–40 | 7 | 12 |
| 41–50 | 2 | 3 |
| 51–60 | 3 | 5 |
| 61–70 | 1 | 2 |
| 71–80 | 3 | 5 |

77% and 67% respectively (Table 2). The resistance to furazolidone, trimethoprim–sulfamethoxazole and erythromycin was 100%, 98% and 62% respectively.

Discussion

The 7th pandemic of El Tor cholera that started in 1961 was still active in 1998

Table 2 Results of antibiogram tests of *Vibrio cholerae* isolates (n = 60) in Hamadan, Islamic Republic of Iran in 2005

| Antibiotic | Sensitivity | | Resistance | |
|--|-------------|----|------------|-----|
| | No. | % | No. | % |
| Norfloxacin | 58 | 97 | 2 | 3 |
| Ciprofloxacin | 55 | 92 | 5 | 8 |
| Kanamycin | 53 | 88 | 7 | 12 |
| Amikacin | 51 | 85 | 9 | 15 |
| Tetracycline | 46 | 77 | 14 | 23 |
| Doxycycline | 40 | 67 | 20 | 33 |
| Erythromycin | 23 | 38 | 37 | 62 |
| Trimethoprim–sulfamethoxazole ^a | 1 | 2 | 39 | 98 |
| Furazolidone | 0 | 0 | 60 | 100 |

^an = 40 specimens only.

when a marked increase was recorded in the number of cases in all countries affected, with a total of 293 121 cases and 10 586 deaths reported to the World Health Organization [8]. This is almost double the number of reported cases in 1997 (147 425 cases and 6274 deaths) [8–10].

In the present study in Hamadan province in the west of the Islamic Republic of Iran in 2005, 190 isolates out of 13 172 were identified as *V. cholerae* O1, biotype El Tor and serotype Inaba. In their study of the 1998 cholera epidemic in Hamadan, Keramat et al. reported 718 isolates as *V. cholerae* O1, biotype El Tor and serotype Ogawa [11].

Al-Abbassi et al. reported an epidemic of cholera in Baghdad, Iraq during 1999 from which 874 strains isolated were *V. cholerae* El Tor O1, serotypes Ogawa (79.6%) and Inaba (12.1%), *V. parahaemolyticus* (2%) and non-agglutinable vibrios (6.1%). *V. cholerae* O139 was isolated from 2 cases (0.2%) for the first time in Iraq [4]. Kaistha et al. reported an outbreak of cholera in and around Chandigarh, India during 2 successive years (2002 and 2003) in which 99 isolates were found to be *V. cholerae* O1 serotype Ogawa, biotype El Tor [12]. In the Islamic Republic of Iran *V. cholerae* O139 has not yet been isolated in any of the several epidemics.

In our study, the case fatality rate was 1.1%. In the 1998 cholera epidemic in Hamadan, the case fatality rate was 0.7%, less than the global case fatality rate of cholera of 4.3% during 1997 and 3.6% during 1998 [8].

We found that the antibiotic resistance to trimethoprim–sulfamethoxazole, furazolidone and erythromycin were over 60%. Table 3 (from Keramat et al.'s study) shows the results of antibiograms of 100 specimens of *V. cholerae*. Antibiotic resistance to trimethoprim–sulfa-

Table 3 Results of antibiogram tests of *Vibrio cholerae* isolates (n = 100) in Hamadan, Islamic Republic of Iran in 1998 [11]

| Antibiotic | Sensitivity % | Resistance % |
|-------------------------------|---------------|--------------|
| Ciprofloxacin | 92 | 8 |
| Tobramycin | 93 | 7 |
| Doxycycline | 85 | 15 |
| Erythromycin | 73 | 27 |
| Trimethoprim–sulfamethoxazole | 0 | 100 |
| Furazolidone | 0 | 100 |
| Ampicillin | 8 | 92 |
| Nalidixic acid | 98 | 2 |
| Chloramphenicol | 33 | 67 |

Source: Keramat et al. [11].

methoxazole and furazolidone was 99% and 98%, but the sensitivity to ciprofloxacin, doxycycline and erythromycin was 99%, 85% and 73% respectively [11]. In another

study, by Pourshafie et al., 200 isolates of *V. cholerae* were obtained in 1999 and 2000 from patients suspected of having cholera in different provinces of the Islamic Republic of Iran [13]. The antibiotic resistance study showed significant resistance to trimethoprim–sulfamethoxazole, streptomycin and furazolidone but 100% of the strains were sensitive to ciprofloxacin and gentamicin (Table 4). Most *V. cholerae* O1 isolates from different provinces in the Islamic Republic of Iran are characterized by resistance to multiple antibiotics. These studies show an increasing frequency of resistance. The reasons for the variation in antibiotic susceptibility patterns between different provinces are unclear.

Singh et al. reported an epidemic of cholera in Delhi in 1995 where resistance to furazolidone, streptomycin, trimethoprim–sulfamethoxazole, chloramphenicol, nalidixic acid and tetracycline was 95%, 91%, 89%, 8%, 7% and 4% respectively [14].

Table 4 Results of antibiogram tests of *Vibrio cholerae* isolates in different regions of the Islamic Republic of Iran in 1999–2000 [13]

| Antibiotic | Resistance (%) | | | | | |
|-------------------------------|--------------------|--------------------|-----------------------|--------------------|-----------------------|---------------------------------|
| | Tehran (n = 58) | Kashan (n = 29) | Khuzastan (n = 60) | Kerman (n = 44) | Kermanshah (n = 4) | Sistan & Baluchestan (n = 5) |
| Chloramphenicol | 0 | 0 | 2 | 2 | 0 | 0 |
| Ciprofloxacin | 0 | 0 | 0 | 0 | 0 | 0 |
| Doxycycline | 0 | 0 | 0 | 5 | 0 | 0 |
| Erythromycin | 0 | 100 | 0 | 2 | 0 | 0 |
| Tetracycline | 100 | 0 | 95 | 2 | 100 | 0 |
| Trimethoprim–sulfamethoxazole | 100 | 0 | 97 | 100 | 100 | 100 |
| Tetracycline | 0 | 0 | 27 | 5 | 0 | 0 |
| Gentamicin | 0 | 0 | 0 | 0 | 0 | 0 |
| Furazolidone | 100 | 0 | 34 | 100 | 100 | 100 |
| Streptomycin | 100 | 100 | 100 | 100 | 100 | 100 |

n = no. of isolates.

Another study by Sow et al. showed that *V. cholerae* O1 strains were multiresistant to sulfonamide, trimethoprim-sulfamethoxazole and chloramphenicol but fluoroquinolone and 3rd generation cephalosporins were more effective antibiotics (100% resistant) [15]. Other investigators have reported *V. cholerae* O1 that is drug-resistant to tetracycline, ampicillin, erythromycin, chloramphenicol, nalidixic acid and trimethoprim-sulfamethoxazole [16,17]. Gabastou et al. described the outbreak of cholera in Ecuador in 1998 and reported 100% of 301 strains of *V. cholerae* were sensitive to tetracycline and quinolones, and 5.6% of the strains were resistant to erythromycin [18].

Antimicrobial agents play a secondary role (after rehydration) in the treatment of cholera. Clinical trials have shown that when patients are given antibiotics the duration of diarrhoea decreases and the volume of stool reduces. These benefits are critical in epidemic conditions [2]. Oral tetracycline and doxycycline are the agents of choice in areas of the world where sensitive strains predominate. In children younger than 7 years trimethoprim-sulfamethoxazole, erythromycin and furazolidone are preferred. Pregnant women can be treated with erythromycin or furazolidone [2,19-21]. New agents have been tested in endemic and epidemic areas, with quinolones (such as ciprofloxacin, norfloxacin) being the most effective. Quinolones have not been recommended for children < 18 years and pregnant women [1,2,22]. In the present

study, ciprofloxacin resistance was 8%, the same as in the 1998 Hamadan epidemic.

However, strains resistant to quinolones have recently been reported from India [2,23-25]. Early planning to contain the disease may have reduced the case fatality rate, especially through proper regimens of rehydration and antibiotic therapy. Studying the antibiograms of the 2 epidemics of cholera in Hamadan and other provinces of the Islamic Republic of Iran reveals that resistance to trimethoprim-sulfamethoxazole and furazolidone has remained high, while resistance to erythromycin, doxycycline and ciprofloxacin has been increasing. This problem will cause some difficulties concerning children and pregnant women with cholera.

Our study suggests that there is a great need for the Ministry of Health and Medical Education in our country to control the utilization of antimicrobial agents in cholera, in addition to carrying out surveillance of antimicrobial resistance as a guide to the choice of antimicrobial for treatment.

Acknowledgements

The authors thank Mr Saeid Ejmalian and Ms Zohre Zarei-Ghane in the Reference Laboratory of the Health Centre, Hamadan for carrying out tests. We also thank Dr Mohammad Reza Honarvar and his other colleagues of the health office in Hamadan for their help in carrying out this research.

References

1. Waldor MK et al. Cholera and other vibrios. In: Kasper DL et al., eds. *Harrison's principles of internal medicine*, 16th ed. New York, McGraw-Hill, 2005:909-12.
2. Seas C et al. *Vibrio cholerae*. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and practice of infectious diseases*, 6th

- ed. Philadelphia, Churchill Livingstone, 2005:2536–44.
3. Yamamoto T et al. Survey of *in vitro* susceptibilities of *Vibrio cholerae* O1 and O 139 to antimicrobial agents. *Antimicrobial agents and chemotherapy*, 1995, 39:241–4.
 4. Al-Abbassi AM, Ahmed S, Al-Hadithi T. Cholera epidemic in Baghdad during 1999: clinical and bacteriological profile of hospitalized cases. *Eastern Mediterranean health journal*, 2005, 11(1/2):6–13.
 5. Farme J et al. The prokaryotes. In: Blaws AG et al, eds. *Handbook on the biology of bacteria: ecophysiology, isolation, identification, applications*, 2nd ed. New York, Springer-Verlag, 1992:2952–3011.
 6. Tison D. *Vibrio*. In: Murray PR, Barron EJ, eds. *Manual of clinical microbiology*. Washington DC, ASM Press, 1999:497–506.
 7. Bauer AW et al. Antibiotic susceptibility testing by standardized single disc method. *American journal of clinical pathology*, 1996, 45:493–6.
 8. Cholera, 1998. *Weekly epidemiological record*, 1999, 74(31):257–64.
 9. Cholera, 1999. *Weekly epidemiological record*, 2000, 75(31):249–26.
 10. Cholera, 2000. *Weekly epidemiological record*, 2001, 76(31):233–40.
 11. Keramat F et al. [A survey of etiologic, clinical manifestations and laboratory findings of patients with cholera in the Hamadan province epidemic in 1998]. *Journal of Hamadan University of Medical Sciences*, 2003, 10(3):43–50 [in Farsi].
 12. Kaistha N et al. Outbreak of cholera in and around Chandigarh during two successive years (2002, 2003). *Indian journal of medicine research*, 2005, 122:404–7.
 13. Pourshafie M et al. A molecular and phenotypic study of *Vibrio cholerae* in Iran. *Journal of medical microbiology*, 2002, 51:392–8.
 14. Singh J et al. Endemic cholera in Delhi 1995: analysis of data from a sentinel center. *Journal of diarrhoeal diseases research*, 1998, 16(2):66–73.
 15. Sow AI et al. Diversite bacterienne au cours de l'epidemie de cholera a Dakar, Senegal (1995–1996) [Bacterial diversity during the cholera epidemic in Dakar, Senegal (1995–1996)]. *Bulletin de la Societe de pathologie exotique*, 1997, 90(3):160–1.
 16. Sabeen F et al. *In vitro* susceptibility of *Vibrio cholerae* O1 biotype El Tor strains associated with an outbreak of cholera in Kerala, Southern India. *Journal of antimicrobial chemotherapy*, 2001, 47:361–2.
 17. Urassa WK et al. Antimicrobial susceptibility pattern of *Vibrio cholerae* O1 strains during two cholera outbreaks in Dar es Salaam, Tanzania. *East African medical journal*, 2000, 77(7):350–3.
 18. Gabastou JM et al. Caracteristicas de la epidemia de colera de 1998 en Ecuador, durante el fenomeno de “El Nino” [Characteristics of the cholera epidemic of 1998 in Ecuador during El Nino]. *Revista panamericana de salud publica*, 2002, 12(3):157–64.
 19. Garg P et al. Emergence of fluoroquinolone resistant strains of *Vibrio cholerae* O1 among hospitalized patient in Calcutta, India. *Antimicrobial agents and chemotherapy*, 2001, 45:1605–6.
 20. Greenough WB et al. Tetracycline in the treatment of cholera. *Lancet*, 1964, 1:355.
 21. O'Grady EM et al. *Global surveillance of antibiotic sensitivity of Vibrio cholerae*. *Bulletin of the World Health Organization*, 1976, 54:181.
 22. Mukhopadhyay AK et al. Biotype traits and antibiotic susceptibility of *Vibrio cholerae* serogroup O1 before, during and after the emergence of the O139

- serogroup. *Epidemiology and infection*, 1995, 115:427–34.
23. Hooper DC et al. The fluoroquinolones: pharmacology, clinical uses, and toxicities in humans. *Antimicrobial agents and chemotherapy*, 1985, 28:716–21.
24. Mukhopadhyay AK et al. Emergence of fluoroquinolone resistance in strains of *Vibrio cholerae* isolated from hospitalized patients with acute diarrhea in Calcutta, India. *Antimicrobial agents and chemotherapy*, 1998, 42:206–7.
25. Bhattacharya MK et al. Outbreak of cholera caused by *Vibrio cholerae* O1 intermediately resistant to norfloxacin at Malda, West Bengal. *Journal of the Indian Medical Association*, 2000, 98(7):389–90.

Practical guide on environmental health in emergencies and disasters

The Centre for Environmental Health Activities has just published the Arabic version of the WHO publication "Environmental health in emergencies and disasters: practical guide". This document distills what is known about environmental health (EH) during an emergency or disaster. It is intended for practitioners, as well as for policy-makers and researchers. In part I, a conceptual framework is presented for understanding EH issues in the context of disaster management. The framework covers the entire disaster-management cycle. Guidelines are also suggested for planning and reducing the effects of extreme events on public health. Part II of this book is a detailed compendium of best practices and strategies for risk reduction and response. Topics covered include: shelter and emergency settlements; water supply; sanitation; food safety; vector and pest control; prevention of epidemics; chemical incidents; radiation emergencies; and mortuary services and handling of the dead. The complete document can be accessed online at: <http://www.emro.who.int/ceha/pdf/EHemerg.pdf>