per mice was 200 mg and the dilution used for inoculating agar plates, the lower limit of detection was $5\$10^2$ CFU/g. For 15 mice, no AB was recovered into the lungs. Therefore, the number of surviving bacteria was under this limit of detection. For the 2 remaining mice, the bacterial counts were $6.17\$10^2$ CFU/g and $1.43\$10^4$ CFU/g respectively. The MIC of rifampicin for surviving bacteria did not change and was still 3 mg/L, as before treatment.

Some studies demonstrated the interest of using rifampicin in combination with other antibiotics for the treat-ment of CRAB. Rifampicin was efficacious for the treatment of severe infections (murine model of pneumonia and rabbit model of meningitis) due to a CRAB strain, in combination with imipenem or sulbactam to prevent the development of resistance.[4] In another study using an im-munosuppressed mouse model of CRAB pneumonia, rifampicin-based combinations with imipenem or colistin were effective by reducing the bacterial loads in lungs and against bacteraemia.[5] A more recent study[6] also demonstrated a synergic effect between meropenem and rifampicin in a murine model of sepsis caused by a CRAB. However, another study[7] demonstrated that in vitro syner-gism or an additive interaction between rifampicin and imi-penem most likely occurs in AB strains showing moderate resistance to imipenem (MIC 64 mg/l). The authors considered that using this combination in the therapy of in-fections caused by strains with higher levels of resistance was not recommended since imipenem could not prevent the development of rifampicin resistance.

In our study, two results must be considered. First, colistin used as a single agent did not show any efficacy in our model of pneumonia. This result could be explained in part by the weak diffusion of colistin in lungs and by the compartmentalised nature of our model (bacterial counts 10^5 fold higher in lungs than in blood).[3] Concurrently, the mice survival rate in the rifampicin group was dramatically high and according to the methodology that we used, no rifampicin-resistant mutant was selected. This could be explained by the high rifampicin concentrations that can be achievable in lungs.

Additional experiments including the determination of intra-pulmonary concentrations of antibiotics are needed for a more complete evaluation of the possibility of using rifampicin in monotherapy in last resort when the MICs of carbapenems are very high (>64 mg/l).

References

1. Vardakas KZ, Rafailidis PI, Konstantelias AA, Falagas ME. Predictors of mortality in patients with infections due to multi-drug resistant Gram negative bacteria: the study, the patient, the bug or the drug? J Infect 2013;66:401e14.
2. Kempf M, Rolain JM. Emergence of resistance to carbapenems in Acinetobacter baumannii in Europe: clinical impact and therapeutic options. Int J Antimicrob Agents 2012;39:105e14.
3. Eveillard M, Soltner C, Kempf M, Saint-Andre JP, Lemarie C, Randrianarivelo C, et al. The virulence variability of different Acinetobacter baumannii strains in experimental pneumonia. J Infect 2010;60:154e61.
4. Pachon-Ibanez ME, Docobo-Perez F, Lopez-Rojas R, Dominguez-Herrera J, Jimenez-Meijas ME, Garcia-Curiel A, et al. Efficacy of rifampicin and its combinations with imipenem, sulbactam,a nd colistin in experimental models of infection caused by imipenem-resistant Acinetobacter baumannii. Antimicrob Agents Chemother 2010;54:1165e72.
5. Song JY, Cheong HJ, Lee J, Sung AK, Kim WJ. Efficacy of monotherapy and combined antibiotic therapy for carbapenem-resistant Acinetobacter baumannii pneumonia in an immunosup-pressed mouse model. Int J Antimicrob Agents 2009;33:33e9.
6. Sun Y, Wang L, Jiankang L, Chongbo Z, Jinyi Z, Meiyou L, et al. Synergistic efficacy of meropenem and rifampicin in a murine model of sepsis caused by multidrug-resistant Acinetobacter baumannii. Eur J Pharmacol 2014;729:116e22.
7. Majewski P, Ojdana D, Sacha PT, Wieczorek A, Tryniszewska EA. In vitro activity of rifampicin alone and in combination with imipenem against multidrug-resistant Acinetobacter baumannii harbouring the bla_{OxA-72} resistance gene. Scand J Infect Dis 2014;46:260e4.

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Low coverage and acceptable effectiveness of single dose of Japanese encephalitis vaccine, Gorakhpur division, Uttar Pradesh, India, 2013

Sir,

Ergunay and colleagues reported flavi-viruses (West Nile, tick borne encephalitis and Toscana) to be important etiological agents of meningoencephalitis in Turkey.[1] In India, Japanese encephalitis (JE) is a leading cause

of acute encephalitis. During 2008e2013, more than 4500 laboratory confirmed cases were reported from 20 Indian states with 805 (17.5%) deaths.[2] About one-third of these cases were from the state of Uttar Pradesh.[1] In Gorakhpur division of Uttar Pradesh, seasonal outbreaks of Acute Encephalitis Syndrome (AES) are frequent.[3e5] During 2008e2012, more than 10,000 AES cases were reported from Gorakhpur division, 8% of which were due to JE.[5] JE incidence was highest among under-five children. The case fatality ratio due to JE during this period was 20%.[6]

Vaccination is the most cost-effective strategy for preventing JE. Following a large outbreak in 2005,[5] the Chinese live attenuated vaccine (SA 14-14-2 strain) was introduced for JE control in the Gorakhpur division. Mass vaccination with single dose of JE vaccine targeting children aged 1e15 years was conducted in 2006 and 2010. The evaluated coverage of 2006 campaign was low (52%) whereas no information is available about the coverage during 2010 campaign.[7] In 2011, JE vaccine was introduced in the childhood immunization programme as a single dose targeting children between 16 and 24 months along with DPT/OPV booster. In 2013, a two-dose JE vaccination strat-egy was introduced, with first dose given between 9 and 12 months along with measles vaccine.

Information about the coverage and effectiveness of JE vaccine is necessary to guide the vaccination programme. Although the surveillance data indicated a decline in JE incidence in Gorakhpur division since 2010,[6] reliable estimates of coverage and effectiveness are not available. We conducted a study to estimate the coverage and effectiveness of single dose of JE vaccine administered under routine immunization in Gorakhpur division.

To estimate the JE vaccine coverage, we conducted a cross-sectional survey among children aged between 24 and 54 months in all the four districts (Gorakhpur, Deoria, Kushinagar and Maharajganj) of Gorakhpur division. We needed a sample size of 768 per district assuming vaccination coverage of 20%,[8] absolute precision of 4% and design effect of 2. We sampled 40 clusters (villages in rural areas, wards in urban areas) from each district using probability proportional to their sizes and selected 20 children aged between 24 and 54 months from each cluster. After obtaining written informed consent, mothers of the selected children were interviewed about JE vaccination. A child was considered as vaccinated if he/she had received one dose of JE vaccine between 16 and 24 months of age as per the vaccination card or based on mother's history.

We surveyed 3200 children from Gorakhpur division, of whom 51% were males, 23% belonged to scheduled caste/tribe and 56% had vaccination cards (Supplementary Table 1). The coverage of JE vaccine in the division was 51% (95% CI: 47.9e54.2); ranging between 36% in Kushinagar to 66.0% in Gorakhpur district (Table 1). The coverage was not different by gender or caste. The common reasons cited by 1567 mothers for non-vaccination of their children included lack of information about JE vaccination (75%) and obstacles for vaccination (56%) (Supplementary Table 2).

To estimate JE vaccine effectiveness (VE), we conducted an unmatched case-control study among children aged 24e54 months. Assuming 50% coverage of JE vaccine, odds ratio of 0.18,[9] 5% alpha error and 90% power, we needed 31 cases and 62 controls. AES case-patients aged between 24 and 54 months with laboratory confirmed JE infection based on IgM antibodies in serum and/or CSF, admitted in the Baba Raghav Das Medical College, Gorakh-pur e the only tertiary care hospital in the region, were considered as cases. We selected two healthy controls aged between 24 and 54 months from the same village as that of the case. Mothers of cases and controls were inter-viewed to collect information about JE vaccination.

We included 33 cases and 66 controls in the case-control study. About 75% JE cases and 48% controls were aged >36 month (Table 2). The age and gender adjusted odds ratio associated with JE vaccination was 0.16 (95% CI: 0.05e0.47). The effectiveness of the vaccine was 84% (95% CI: 53e95).

We also compared the vaccination status of 33 JE cases and 3200 children surveyed for the estimation of JE vaccine coverage. Six (18%) JE cases and 1633 (51%) healthy controls had received JE vaccine. The odds ratio associated with JE vaccination was 0.21 (95% CI: 0.08e0.54). The effective-ness of vaccine was 79% (95% CI: 46e92).

The findings of our survey indicated that only half of the eligible children in Gorakhpur division had received one dose of JE vaccine. In spite of frequent outbreaks of JE/AES in the area, majority of the mothers of unvaccinated children were not aware about the need for JE vaccine. While the decline in the incidence of JE in Gorakhpur, as reflected from the surveillance data could be on account of JE vaccination initiatives, the overall coverage of JE vaccine in the area was low.

The occurrence of JE cases in spite of mass vaccination campaigns and introduction of the vaccine in the routine immunization programme have raised doubts among the public health authorities in the region about the effectiveness of the vaccine. In Lucknow, Uttar Pradesh, the effectiveness of single-dose vaccination strategy within six months of introduction of vaccine was found to be 95%.[9] The findings of our study indicated that the effective-ness of JE vaccine, two years after its introduction in

Table 1 Coverage (%) of Japanese encephalitis vaccine by district, Gorakhpur division, 2013.

District	Number of children surveyed	Percentage of children vaccinated (95% CI)		
		As per vaccination card	As per mother's history	Total
Deoria	800	33.6 (28.9e55.4)	16.4 (12.8e20.7)	50.0 (44.6e55.4)
Kushinagar	800	23.3 (18.1e29.4)	12.8 (9.8e16.5)	36.1 (29.2e43.4)
Gorakhpur	800	33.5 (27.4e40.2)	32.5 (27.1e38.4)	66.0 (58.0e73.2)
Maharajganj	800	34.3 (27.4e41.8)	17.9 (14.2e22.3)	52.2 (47.9e54.2)
Gorakhpur division	3200	31.2 (28.4e34.1)	19.9 (17.9e22.0)	51.1 (47.9e54.2)

Table 2 Demographic details and vaccination status of Japanese encephalitis cases and controls, Gorakhpur and Basti Division, Uttar Pradesh, 2013.

Characteristics	Cases (n Z 33)	Control (n Z 66)	Univariate odds ratio (95% CI)	P	Adjusted odds ratio (95% CI)
Age group (months)					
24e36	8	34	1		
37e54	25	32	3.32 (1.31e8.42)	0.012	4.0 (1.5e10.9)
Gender					
Female	12	37	1		
Male	21	29	2.23 (0.94e5.28)	0.067	
Caste					
General/OBC	24	46	1		
Scheduled caste/tribe	9	20	0.86 (0.34e2.18)	0.755	
District					
Basti division	16	32	1		
Gorakhpur division	17	34	1 (0.43e2.31)	1.00	
Presence of vaccination card					
Yes	6	38	1	0.000	
No	27	28	6.1 (2.22e16.77)		
History of JE vaccination					
Not vaccinated	27	31	1		
Vaccinated	6	35	0.20 (0.07e0.54)	0.002	0.16 (0.05e0.4)
Vaccinated as per vaccination card	2	22			
Vaccinated as per mother's history	4	13			

the programme was comparable to the studies conducted in Nepal and China using single-dose of SA 14-14-2 vaccine.[10e13]

There is an urgent need to improve the coverage of JE vaccine in Gorakhpur division. In order to achieve high population immunity before the next transmission season beginning in July, as well as in view of high attack rates among under-five children, health authorities might consider conducting another mass vaccination campaign targeting children aged 1e5 years. It is also necessary to make mothers aware about the need to administer two doses of JE vaccine to their children, which are given free of cost in all the public health facilities. Public health managers also need to be assured that the JE vaccine currently used in the programme has acceptable effectiveness.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jinf.2014.06.017.

References

1. Ergunay K, Sayiner AA, Litzba N, Lederer S, Charrel R, Kreher P, et al. Multicentre evaluation of central nervous system infections due to Flavi and Phleboviruses in Turkey. J Infect 2012; 65:343e9.
2. Directorate of National Vector Borne Diseases Control Programme. Details of AES/JE cases and deaths from 2008e2014. Available at: http://www.nvbdcp.gov.in/Doc/je-aes-till-12May14.pdf.
3. Kar NJ, Bora D, Sharma RC, Bhattacharjee J, Datta KK, Sharma RS. Epidemiological profile of Japanese encephalitis in Gorakhpur district, Uttar Pradesh, 1982e1988. J Commun Dis 1992;24:145e9.
4. Kumari R, Joshi PL. A review of Japanese encelpahlitis in Uttar Pradesh, India. WHO Southeast Asia J Public Health 2012;1: 374e95.
5. Kumar R, Tripathi P, Singh S, Bannerji G. Clinical features in children hospitalized during the 2005 epidemic of Japanese encephalitis in Uttar Pradesh, India. Clin Infect Dis 2006;43:123e31.
6. Ranjan P, Gore MM, Selvaraju S, Kushwaha KP, Srivastava DK, Murhekar MV. Changes in acute encephalitis syndrome inci-dence after introduction of Japanese encephalitis vaccine in a region of India. J Infect 2014;69(2):200e2.
7. Ministry of Health and Family Welfare, Government of India. Japanese encephalitis coverage evaluation survey report. 2008. Available at: http://www.unicef.org/india/Japanese_Encephalitis_CES_2008_report.pdf.
8. Ministry of Health and Family Welfare, Government of India. Coverage evaluation survey, Uttar Pradesh fact sheet. 2009. Available at: http://www.unicef.org/india/Uttar_Pradesh_Fact_Sheet.pdf.
9. Kumar R, Tripathi P, Rizvi A. Effectiveness of one dose of SA 14-14-2 vaccine against Japanese encephalitis. N Eng J Med 2009; 360:1465e6.

10. Bista MB, Banerjee MK, Shin SH, Tandan JB, Kim MH, Sohn YM, et al. Efficacy of single-dose SA 14e14e2 vaccine against Japanese encephalitis: a case control study. Lancet 2001; 358:791e5.
11. Ohrr H, Tandan JB, Sohn YM, Shin SH, Pradhan DP, Halstead SB. Effect of single dose of SA 14-14-2 vaccine 1 year after immu-nisation in Nepalese children with Japanese encephalitis: a case-control study. Lancet 2005;366:1375e8.
12. Tandon JB, Ohrr H, Sohn YM, Yoksan S, Ji M, Nam CM, et al. Sin-gle dose of SA 14-14-2 vaccine provides long-term protection against Japanese encephalitis: a caseecontrol study in Nepal-ese children 5 years after immunization. Vaccine 2007;25: 5041e5.
13. Hennessy S, Liu Z, Tsai TF, Strom BL, Wan CM, Liu HL, et al. Effectiveness of live-attenuated Japanese encephalitis vaccine (SA14-14-2): a case-control study. Lancet 1996;347:1583e6.

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