Title

Randomised, controlled trial of effectiveness of ampicillin in mild acute respiratory infections in Indonesian children

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the findings obtained in necropsy studies. Information collated from five independent necropsy studies indicated that mean prostate weight reaches 20 g in men between the ages of 21 and 30 years and remains essentially constant at this weight with increasing age unless BPH develops.[10] Table II summarises the prevalence rates of BPH and prostatic weights of patients with BPH in the survey in comparison with necropsy findings among men in similar age-groups with histologically confirmed BPH. A surprising finding is that prostatic weights from these two sources are remarkably similar despite the differences in self-selection betwen the community survey and necropsy sources, the absence of prior knowledge of urinary dysfunction in the necropsy cases, and the absence of pathological confirmation of the diagnosis in the community survey. What is also of interest are the higher age-specific rates for BPH in the necropsy than in the community survey. This difference suggests that a substantial reservoir of BPH may exist below the "threshold" of signs and symptoms of urinary dysfunction that were used in the survey. The operational definition adopted for this survey will probably change over time as knowledge of the natural history of BPH increases, in the way that perception of what level of blood pressure constitutes hypertension has changed over the past several decades.

Whether the total urinary symptom score and Q_{max} cut-off points used are ideal for enabling early cases of BPH to be picked up through screening cannot be established from this survey. This issue can be determined only in a study that sets out to assess prostate size in a representative sample of men who have not been selected on the basis of their likelihood of having BPH. The validity of symptom scores and voiding flow rate as preliminary screening criteria will eventually be required for identifying those men most likely to benefit from treatment should non-surgical therapy for BPH be shown to be effective in the community.

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REFERENCES

1. Graves EJ. Detailed diagnosis and procedures, National Hospital Discharge Survey, 1987. National Center for Health Statistics, Washington DC. Vital and Health Statistics 1989; **13:** 295.
2. Jensen KM-E, Jorgensen JB, Morgensen P, et al. Some clinical aspects of uroflowmetry in elderly males. A population study. *Scand J Urol Nephrol* 1986; **20:** 93–97.
3. Glynn RJ, Campion EW, Bouchard GR, et al. The development of benign prostatic hyperplasia among volunteers in the Normative Aging Study. *Am J Epidemiol* 1985; **121:** 78–90.
4. Meyhoff HH, Hald T. Are doctors able to assess prostatic size. *Scand J Urol Nephrol* 1978; **12:** 219–21.
5. Fowler FJ, Wennberg JE, Timothy RP, et al. Symptom status and quality of life following prostatectomy. *JAMA* 1988; **259:** 3018–22.
6. Rowan D, McKenzie AL, McNee SG, et al. A technical and clinical evaluation of the Disa uroflowmeter. *Br J Urol* 1977; **49:** 285–91.
7. Griffiths CJ, Murray A, Ramsden PO. A simple uroflowmeter tester. *Br J Urol* 1983; **55:** 21–24.
8. Jensen KM-E, Jorgensen JB, Morgensen P. Reproducibility of uroflowmetry variables in elderly males. *Urol Res* 1985; **13:** 237–39.
9. Abu-Yousef MM, Narayana AS. Transabdominal ultrasound in the evaluation of prostate size. *J Clin Ultrasound* 1982; **10:** 275–78.
10. Berry SJ, Coffey DS, Walsh PC, et al. The development of human benign prostatic hyperplasia with age. *J Urol* 1984; **132:** 474–79.
11. Watanabe H. Natural history of benign prostatic hypertrophy. *Ultrasound Med Biol* 1986; **12:** 567–71.

Randomised, controlled trial of effectiveness of ampicillin in mild acute respiratory infections in Indonesian children

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The recommended treatment for mild acute respiratory infections (ARI) in children is supportive care only, but many physicians, especially in developing countries, continue to prescribe antibiotic treatment because they believe it prevents progression to more severe ARI. To find out whether ampicillin treatment conferred any benefit over supportive care alone, a randomised, controlled trial was carried out among 889 children (under 5 years) with mild ARI in Indonesia. 447 were randomly allocated ampicillin (25–30 mg/kg body weight three times daily for 5 days) plus supportive care (continued breastfeeding, clearing of the nose, and paracetamol to control fever); 442 were allocated supportive care only. The treatment groups were almost identical after randomisation in terms of age, sex, level of parental education, history of measles immunisation, and fever. After 1 week the percentages cured were nearly identical (204 [46%] ampicillin; 209 [47%] control), as were the percentages of cases progressing to moderate ARI (56 [13%] *vs* 53 [12%]). The effect of treatment was not modified by age, sex, measles immunisation status, or the educational level of the parents. At the 2-week follow-up, the percentages cured were 62% (277) in the ampicillin group and 58% (256) in the control group; 14% of both groups had progressed to moderate ARI; and 24% (107) and 28% (123), respectively, still had mild ARI. None of the differences in outcome between the ampicillin and control groups was statistically significant. Thus, ampicillin plus supportive care offers no benefit over supportive care alone for treatment of mild ARI in young Indonesian children.

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Introduction

Acute respiratory infections (ARI) are common in children throughout the developing world. The infection

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may start in the upper respiratory tract and progress to a more severe lower respiratory infection, such as pneumonia or bronchiolitis.[1] In less developed countries, pneumonia is a major cause of death in young children .[2-4] Antibiotics such as procaine penicillin, ampicillin, or co-trimoxazole are useful for treatment of pneumonia[1,5,6] which is most commonly caused by bacterial agents, mainly *Streptococcus pneumoniae* and *Haemophilus influenzae*.[1,5,7,8] Antibiotics are not recommended for treatment of upper respiratory infections,[7] which are believed to be caused mainly by viruses, most likely rhinoviruses and coronaviruses.[9]

The World Health Organisation (WHO) has promoted case-management as the appropriate approach to control of ARI in children.[7,10] Part of this recommendation is that supportive care by the mother rather than antibiotics be used to treat mild ARI. Inappropriate treatment of mild ARI in developing countries wastes the resources of government-sponsored health services and is believed to increase the occurrence of drug-resistant bacterial strains in the population.[11]

When the WHO case-management programme was introduced in Indonesia, many physicians were reluctant to stop giving antibiotics to children with mild ARI, because they believed that antibiotic treatment prevents the progression of mild ARI to moderate or severe ARI. This belief is not supported by any objective evidence. We decided to test the effectiveness of antibiotics for treatment of mild ARI in Indonesian children. We now present the results from a randomised, controlled comparison of supportive care alone and ampicillin plus supportive care as treatment for mild ARI in children under 5 years of age. We chose ampicillin on the basis of our earlier findings that 70% of Indonesian physicians who treated mild ARI with antibiotics used ampicillin (unpublished).

Subjects and methods

900 children under 5 years of age with mild ARI, who attended government health clinics in two regions of East Jakarta, Indonesia, were included in the trial. Mild ARI was defined by WHO criteria: mild upper respiratory signs such as cough or runny nose, and/or fever (> 37°C), and breathing at a rate less than 50 breaths per minute. We excluded children with asthma or infections that required antibiotics, those who did not live in a defined service area, and those who had recently been treated by medical personnel in other locations. Parental consent was obtained for all eligible children.

Through random allocation, 451 children were offered ampicillin powder and supportive care and 449 were offered only supportive care. Parents in both groups were advised to provide supportive care at home including continuation of breastfeeding, clearing of the nose as needed, and control of mild fever by means of paracetamol, an inexpensive analgesic and antipyretic drug (30 mg/kg body weight daily; 3 doses per day for 5 days). In addition to the supportive care, children in the ampicillin group received ampicillin powder (age-dependent treatment packets of 25–30 mg/kg body weight per dose every 6 h [except midnight] for 5 days).

Parents were asked to bring their child back to the health centre after 5 days. Home visits were made by nurses or midwives within 2 days to children who did not appear at the clinic on the scheduled day. Based on the clinic or home examination the health status was classified as: cured or recovered; static (no change); worse (moderate or severe ARI); or dead. Moderate and severe ARI were defined according to WHO and Ministry of Health criteria.[7] Moderate ARI was diagnosed if there was a respiratory rate greater than 50 breaths per minute but no chest indrawing, and severe ARI if the child had a respiratory rate greater than 50 breaths per minute and chest indrawing, with or without cyanosis. Children not cured after 1 week were either referred to the health clinic for further medical care or sent home with instructions for additional supportive care. All children were observed by a nurse or midwife 1 week later either at the clinic or at home.

11 children (4 ampicillin, 7 control) had stopped taking ampicillin or paracetamol because of side-effects (diarrhoea in 10 [4 ampicillin, 6 control] and an allergic reaction in 1 [control]).

Statistical analyses were done by means of 'Epi Info'[12] and 'Epi Log Plus',[13] two microcomputer-based statistical analysis programs for epidemiology. These included standard chi-square tests (two sided) and confidence intervals for risk ratios (Taylor series 95% confidence limits).

TABLE I—CHARACTERISTICS OF CHILDREN WITH MILD ARI AT BASELINE

—	No (%)	
	Ampicillin (n = 451)	Control (n = 449)
Age (mo)		
0–11	173 (38)	155 (35)
12–23	118 (26)	121 (27)
24–35	66 (15)	93 (21)
36–59	94 (21)	80 (18)
Sex		
Male	228 (51)	233 (52)
Female	223 (49)	216 (48)
Father's education (yr)		
< 9	135 (30)	151 (34)
9–12	292 (65)	268 (60)
> 12	24 (5)	30 (7)
Mother's education (yr)		
< 9	225 (50)	236 (53)
9–12	220 (49)	205 (46)
> 12	6 (1)	8 (2)
Immunised for measles		
Yes	175 (39)	176 (39)
No	264 (59)	264 (59)
Unknown	12 (3)	9 (2)
Fever at entry		
Yes	395 (87)	398 (89)
No	56 (12)	51 (11)

Results

The randomisation procedure successfully achieved two nearly identical groups in terms of the potential confounding variables of age, sex, level of parental education, history of measles immunisation, or fever at the time of enrolment (table I). After 1 week, the percentages of children who were cured, still had mild ARI, or had progressed to moderate ARI in the two groups were almost identical (table II). There was no significant difference in the course of the disease between the ampicillin-treated and control groups at either 1-week follow-up ($\chi^2 = 0{\cdot}25$, 2 df; $p = 0{\cdot}88$) or 2-week follow-up ($\chi^2 = 1{\cdot}91$, 2 df; $p = 0{\cdot}38$) (table II). After 1 week the percentage cured was similar at all ages, in both sexes, at various education levels for fathers and mothers, and by measles immunisation status (table III). Ampicillin appeared beneficial only among a few subgroups, but in each case the 95% confidence intervals included 1·0, so the risk ratios are more likely to reflect variation inherent in the sampling process rather than a real benefit of ampicillin.

TABLE II—CLINICAL OUTCOME OF MILD ARI

—	No (%)			
	Ampicillin (n = 447)*		Control (n = 442)	
	Day 5–7	Day 14	Day 5–7	Day 14
Cured	204 (46)	277 (62)	209 (47)	256 (58)
Static	187 (42)	107 (24)	180 (41)	123 (28)
Worse (moderate ARI)†	56 (13)	63 (14)	53 (12)	63 (14)

4 ampicillin-treated and 7 control children was excluded during week 1 with secondary disorders

*Received ampicillin for 5 days in wk 1

†Removed from trial and sent to health clinic for further medical care

TABLE III—MILD ARI CASES CURED AT 1-WEEK FOLLOW-UP

	Ampicillin		Control		
—	n	No (%) cured at day 5–7	n	No (%) cured at day 5–7	Risk ratio* (95% CI)
Age (mo)					
0–11	171	65 (38)	155	72 (47)	0·82 (0·63, 1·05)
12–35	182	96 (53)	207	96 (46)	1·14 (0·93, 1·39)
36–59	94	43 (46)	80	41 (51)	0·89 (0·66, 1·21)
Sex					
Male	227	102 (45)	231	107 (46)	0·97 (0·79, 1·18)
Female	220	102 (46)	211	102 (48)	0·96 (0 79, 1·17)
Father's education (yr)					
<9	132	54 (41)	148	81 (55)	0·75 (0·58, 0·96)
⩾9	315	150 (48)	294	128 (44)	1·09 (0·92, 1·30)
Mother's education (yr)					
<9	222	101 (46)	234	112 (48)	0·95 (0·78, 1·16)
⩾9	225	103 (46)	208	97 (47)	0·98 (0·80, 1·20)
Immunised for measles					
Yes	175	91 (52)	172	74 (43)	1·21 (0·97, 1·51)
No	260	109 (42)	261	131 (50)	0·84 (0·69, 1·01)

*Likelihood of being cured in ampicillin group compared with control group; 95% CI = confidence interval
†Excludes 21 cases with unknown measles immunisation history (12 ampicillin, 9 control)

The percentage of cases of mild ARI that progressed to moderate ARI at the 1-week follow-up was also similar in the various subgroups (table IV). In some subgroups the control cases were more likely to progress, whereas in others the ampicillin-treated cases were more likely to become worse. The width of the confidence intervals makes it unlikely that any of the group-specific values are truly different.

After 2 weeks, 24% of ampicillin-treated and 28% of control children still showed signs of mild ARI (table II). 14% in each group had progressed to moderate ARI and were referred to the health clinic for additional care. The remaining 58% of controls and 62% of ampicillin-treated children were cured of ARI, and nearly three-quarters of them had recovered during the first week.

TABLE IV—PROGRESSION TO MODERATE ARI AT 1-WEEK FOLLOW-UP

	Ampicillin		Control		
—	n	No (%) with moderate ARI at day 5–7	n	No (%) with moderate ARI at day 5–7	Risk ratio* (95% CI)
Age (mo)					
0–11	171	19 (11)	155	18 (12)	0·96 (0·52, 1·76)
12–35	182	22 (12)	207	28 (14)	0·89 (0·53, 1·51)
36–59	94	15 (16)	80	7 (9)	1·82 (0·78, 4·25)
Sex					
Male	227	26 (12)	231	29 (13)	0·91 (0·56, 1·50)
Female	270	30 (11)	211	24 (11)	0·98 (0·73, 1·98)
Father's education (yr)					
<9	132	14 (11)	148	14 (10)	1·12 (0·56, 2·26)
⩾9	315	42 (13)	294	39 (13)	1 01 (0·67, 1·51)
Mother's education (yr)					
<9	222	24 (11)	234	28 (12)	0·90 (0 54, 1 51)
⩾9	225	32 (14)	208	25 (12)	1·18 (0·73, 1·93)
Immunised for measles					
Yes	175	17 (10)	172	18 (11)	0·93 (0·50, 1 74)
No	260	37 (14)	261	33 (11)	1·13 (0·73, 1·74)

*Risk of progresion to moderate ARI in ampicillin group compared with control group
†Excludes 21 cases with unknown measles immunisation history (12 ampicillin, 9 control)

Discussion

The randomisation process in our study effectively created two groups with the same inherent risk of ARI outcome independent of treatment. Thus, we were able to assess the unconfounded effect of ampicillin plus supportive care compared with supportive care alone on mild ARI. We conclude that there is no beneficial effect of ampicillin on the clinical course of mild acute respiratory infections among young Indonesian children. For children breathing less than 50 times per minute and showing minor signs such as a cough or runny nose, addition of ampicillin to simple supportive care supplemented with paracetamol conferred no benefit.

Since mild ARI is primarily or entirely caused by viruses, our finding that ampicillin is of no benefit for such illnesses is hardly surprising, and readers might wonder why we undertook this study. In our previous work on ARI in Indonesia (unpublished), we observed that many children with mild ARI were being treated with ampicillin by physicians at Government clinics despite the Ministry of Health guidelines (which accord with WHO recommendations) that only supportive care is required.[14] In our discussions with physicians, it became clear that many believed antibiotics were effective at preventing the progression of mild ARI to pneumonia or other forms of severe ARI, which are frequently bacterial in origin. When challenged to present data refuting this notion, we were unable to find support in the Indonesian medical or public health literature. Thus, we felt it necessary to assess in Indonesia the effect of antimicrobial therapy on progression of mild ARI to more severe forms. Clinical studies of antibiotic effectiveness have been carried out in other nearby countries—for example, Thailand and Australia.[15,16] These studies of children with upper (or mild) respiratory infections showed no therapeutic value for ampicillin, erythromycin, penicillin, or tetracycline.

Although our data clearly show that the use of ampicillin for mild ARI had no beneficial effect, what is not evident is the potential harmful effects of inappropriate antibiotic use. First, ampicillin used for mild ARI will not be available to treat moderate or severe ARI. If there is a limited supply of antibiotics, as is the case in many government health clinics, the resultant shortage could lead to the use of less effective treatment and higher case-fatality for moderate and severe ARI. Even if antibiotics are widely available, use of ineffective treatment for many mild ARI cases will substantially reduce the cost-effectiveness of ARI treatments in general. Second, routine use of antibiotics for common disorders such as mild ARI may contribute to the emergence of drug-resistant bacterial strains, which may become difficult, if not impossible, to treat. In a study of susceptibility of *Haemophilus* species to antimicrobial drugs, 6% of 426 isolates from around the world were resistant to ampicillin.[5] Although this percentage is not great, continued indiscriminate use of antibiotics for minor respiratory ailments can only lead to more resistance.

In summary, our results show that there is no justification for use of ampicillin to treat mild ARI among Indonesian children. This practice is both expensive and potentially harmful and is not in the interests of the medical community, the Ministry of Health, or the Indonesian people.

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REFERENCES

1. Stansfield SK. Acute respiratory infections in the developing world: strategies for prevention, treatment and control. *Pediatr Infect Dis J* 1987; **6:** 622–29.
2. Kalter HD, Gray RH, Black RE, Gultiano SA. Validation of postmortem interviews to ascertain selected causes of death in children. *Int J Epidemiol* 1990; **19:** 380–86.
3. Spika JS, Munshi MH, Wojtyniak B, et al. Acute lower respiratory infections: a major cause of death in children in Bangladesh. *Ann Trop Paediatr* 1989; **9:** 33–39.
4. Denny FW, Loda FA. Acute respiratory infections are the leading cause of death in children in developing countries. *Am J Trop Med Hyg* 1986; **35:** 1–2.
5. Weinberg GA, Spitzer ED, Murray PR, et al. Antimicrobial susceptibility patterns of *Haemophilus* isolates from children in eleven developing nations. BOSTID Haemophilus Susceptibility Study Group. *Bull WHO* 1990; **68:** 79–84.
6. Campbell H, Bypass P, Forgie IM, O'Neill KP, Lloyd-Evans N, Greenwood BM. Trial of co-trimoxazole versus procaine penicillin with ampicillin in treatment of community-acquired pneumonia in young Gambian children. *Lancet* 1988; ii: 1182–84.
7. World Health Organisation. Clinical management of acute respiratory infections in children: a WHO Memorandium. *Bull WHO* 1981; **59:** 707–16.
8. Shann F, Gratten M, Germer S, et al. Aetiology of pneumonia in children in Goroka Hospital, Papua New Guinea. *Lancet* 1984; ii: 537–41.
9. World Health Organisation. Guidelines for research on acute respiratory infections: memorandum from a WHO meeting. *Bull WHO* 1982; **60:** 521–33.
10. World Health Organisation. A program for controlling acute respiratory infections in children: memorandum from a WHO meeting. *Bull WHO* 1984; **62:** 47–58.
11. Kunin CM, Johansen KS, Worning AM, Daschner FD. Report of a symposium on use and abuse of antibiotics worldwide. *Rev Infect Dis* 1990; **12:** 12–19.
12. Dean AD, Dean JA, Burton AH, Dicker RC. Epi Info, version 5: a word processing, database, and statistics program for epidemiology on microcomputers. Stone Mountain, Georgia: USD, Incorporated, 1990.
13. Epi Log Plus, Statistical package for epidemiology and clinical trials. Pasadena, California: Epicentre Software, 1988.
14. Ministry of Health. Health worker manual for ARI case management and diarrhea among children under five. Director General of Communicable Disease Control and Environmental Health, Republic of Indonesia, 1988.
15. Gordon M, Lovell S, Dugdale AE. The value of antibiotics in minor respiratory illness in children—a controlled trial. *Med J Aust* 1974; **9:** 304–06.
16. Lexomboon U, Duangmani C, Kusalasai V, Sunakorn P, Olson LC, Noyes HE. Evaluation of orally administered antibiotics for treatment of upper respiratory infections in Thai children. *J Pediatr* 1971; **78:** 772–78.

SHORT REPORTS

Phylogeny of the Whipple's-disease-associated bacterium

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Efforts to culture and identify the intracellular bacteria associated with Whipple's disease have been unsuccessful. Nucleotide sequencing and amplification by the polymerase chain reaction was done on the bacterial 16 S ribosomal DNA present in a small-bowel biopsy specimen taken from a patient with Whipple's disease. A search by computer for similar rRNA sequences filed in databases showed the Whipple's-associated organism to be most similar to bacteria of the *Rhodococcus, Streptomyces,* and *Arthrobacter* genera, and more weakly related to mycobacteria. The biopsy specimen was estimated to contain around 10^7 cells of the organism. The probable aetiological agent for our patient's illness has not been identified previously in a patient with Whipple's disease.

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Whipple's disease is a systemic infection associated with a small, largely intracellular bacillus of uncertain identity. The nature of the Whipple's bacillus has not been established because efforts to culture the organism have been unsuccessful. We report the results of sequencing the ribosomal DNA of the predominant bacterium associated with a biopsy taken from the small bowel of a woman with Whipple's disease. Comparison of 16 S rRNA sequences is a powerful approach to phylogenetic analysis, and had led to a new phylogenetic tree of all life forms.[1] It was thus possible to place the Whipple's-associated bacterial organism (WABO) phylogenetically by comparing its 16 S rRNA sequence with known sequences.

A 70-year-old woman presented with a 1-year history of diarrhoea, a 9 kg weight loss, and iron-deficiency anaemia. She complained of abdominal distension, arthralgia, fatigue, and myalgia. On physical examination the patient was cachectic with a distended abdomen and thickening of the metacarpals and wrists. X-ray examination of the small bowel suggested malabsorption, and an abdominal computed tomography scan revealed paracaval and periaortic lymphadenopathy. Upper gastrointestinal endoscopy showed a bowel mucosa with a granular infiltrated pattern. Histologically, the small bowel lamina propria was expanded by foamy macrophages and had prominent lymphatic dilatation. Material in the macrophages stained periodic acid-Shiff-positive and did not contain acid-fast organisms. Electron microscopy showed large numbers of intracellular and extracellular bacilli characteristic of those found in Whipple's disease.

After informed consent, an endoscopic biopsy specimen of the proximal small bowel was taken and frozen rapidly in dry ice-ethanol. Nucleic acids extracted from the biopsy specimen were amplified in a polymerase chain reaction (PCR) for 35 cycles. The PCR primers were designed to amplify specifically a 721-base segment of bacterial 16 S rDNA (identical to an organism's rRNA sequence).[2] The resulting PCR product was sequenced directly. A computer search of the Genbank and EMBI databases was done to find the rRNA sequences most similar to the one we had isolated, and all sequences were aligned. The alignment was used to find a site at which the sequence of the WABO differed from the