Clinically and microbiologically derived azithromycin susceptibility breakpoints for *Salmonella enterica* serovars Typhi and Paratyphi A

Christopher M Parry, Tran Vu Thieu Nga, Christiane Dolecek, Abhilasha Karkey, Ruchi Gupta, Paul Turner, David Dance, Rapeephan R Maude, Ha Vinh, Nguyen Tran Chinh, Le Thi Phuong, Pham van Be Bay, Tran Thi Phi La, Nguyen Ngoc Rang, Aniruddha Ghose, Sabina Dongol, James I Campbell, Duy Pham Thanh, Tuyen Ha Thanh, Catrin E Moore, Soeng Sona, Rajni Gaind Monorama Deb, Vo Anh Ho, Nguyen Van Sach, Tran Tinh Hien, Nicholas PJ Day, Arjen Dondorp, Guy Thwaites, Mohamed Abul Faiz, Rattanaphone Phetsouvanh, Paul Newton, Buddha Basnyat, Jeremy J Farrar, Stephen Baker

oucru

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Background

Antimicrobial resistance is a global problem in *Salmonella enterica* serovar Typhi and serovar Paratyphi A

The azalide (macrolide) antimicrobial azithromycin is an effective treatment for uncomplicated enteric fever

There are no clinically validated minimum inhibitory concentration (MIC) and disc zone size interpretative guidelines

EUCAST has suggested MIC ≤16µg/mL as susceptible.

CLSI until recently no recommendation

Aims

Determine the relationship between azithromycin treatment response and the azithromycin MIC of the infecting isolate

Examine the distribution of azithromycin MICs in different Asian countries

Determine the relationship between azithromycin MIC and disc inhibition zone size

Methods

1. We sought to obtain individual patient data from randomised controlled trials (RCTs) of antimicrobial treatment in enteric fever where azithromycin had been used in one of the treatment arms

   Examine the relationship between azithromycin treatment response and the azithromycin MIC of the infecting isolate

2. We compiled azithromycin MIC and the disc inhibition zone sizes of *Salmonella enterica* Typhi and Paratyphi A clinical isolates collected from seven Asian countries

   Determine the MIC distribution in different centres and examine the relationship between azithromycin MIC and 15µg Azithromycin disc inhibition zone size

Results

Seven RCTs published

Three RCTs included in the analysis

Individual patient data only available for the three conducted in southern Vietnam

Chinh NT et al. Antimicrob Agents Chemother 2000; 44: 1855-1859
Azithromycin 1gm orally once daily for 5 days (Mean of 20 mg/kg/day) adults

Parry CM, et al. Antimicrob Agents Chemother. 2007; 51:819-825
Azithromycin 10mg/kg/day orally once a day (maximum 500 mg daily) for 7 days in adults and children

Dolecek C, et al. PLoS ONE 2008.;3(5): e2188
Azithromycin 20 mg/kg/day orally once a day (maximum 1 gm daily) for 7 days in adults and children

Results

There were 248 culture positive patients randomized to azithromycin

In 34 of these patients the bacterial isolate was not available for re-checking the azithromycin MIC

214 patients eligible for analysis

Median age 13 years (IQR 8-20; range 1-68)

Median duration of illness prior to admission of 8 days (IQR 6-10, range 2-30). Infecting isolate was Typhi in 209 patients and Paratyphi A in 5 patients.

137 (64%) were MDR

184 (86%) had intermediate susceptibility to ciprofloxacin

None were ciprofloxacin or ceftriaxone resistant.

The median (IQR, range) azithromycin MIC was 8 µg/mL (8-12, 4-16).

Results

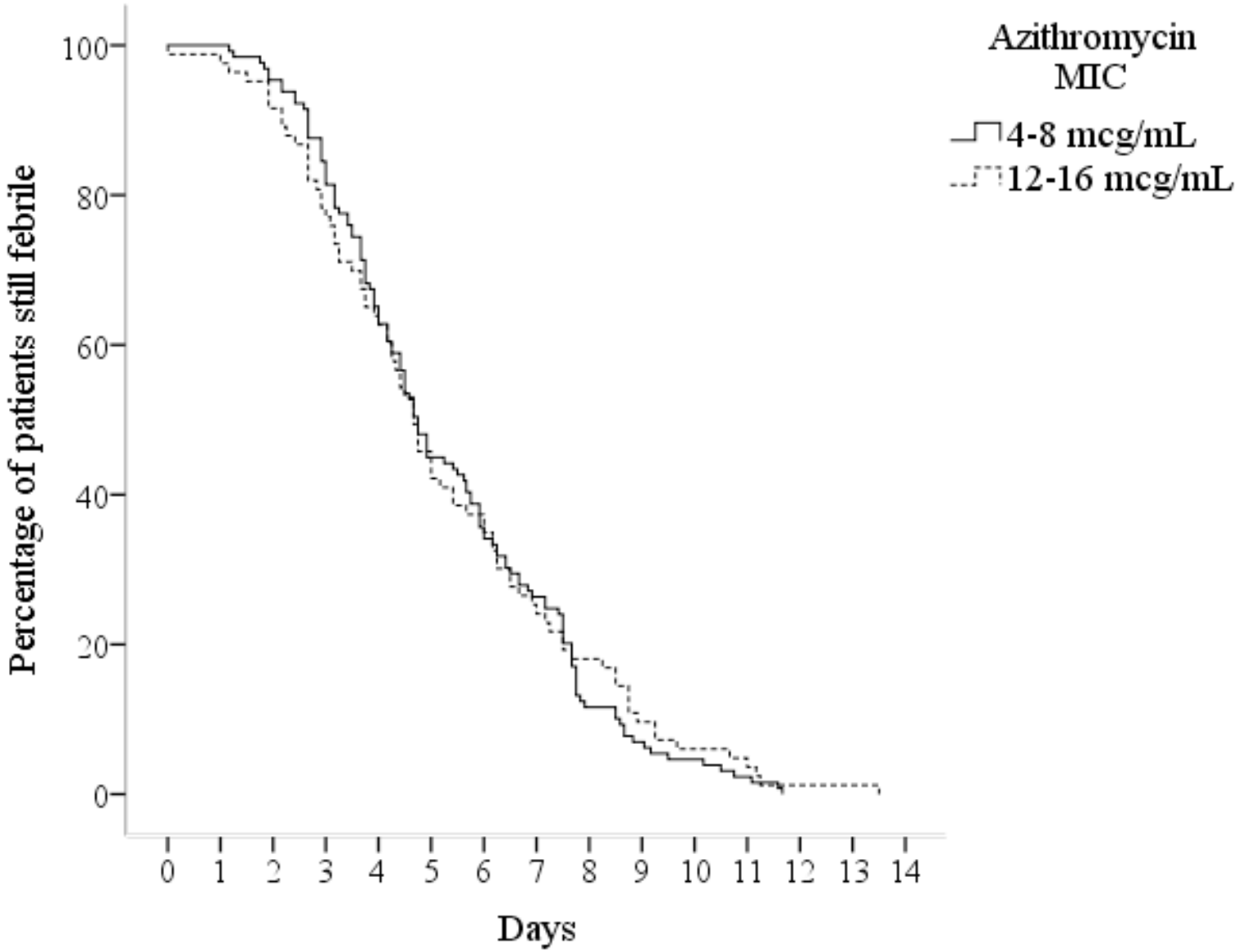
The demographic, clinical and laboratory features of the groups classified by the azithromycin MIC of the infecting isolate were comparable

Treatment was successful in 195/214 (91%; 95% CI, 86.3 to 94.4)

No significant differences in response (cure rate, fever clearance time) according to the azithromycin MIC

Variable	Azithromycin MIC			*p*-value
	4 µg/mL	6-8 µg/mL	12-16 µg/mL	
Number	13	116	85	-
Days till fever clearance time (IQR) (days)	4.4 (3.7-4.5)	4.9 (3.4-7.5)	4.7 (3.2-7.0)	0.249
Any failure (%)	1 (8)	9 (8)	9 (11)	0.775
Clinical failure (%)	1 (8)	9 (8)	8 (9)	0.912
Microbiological failure (%)	1 (8)	1 (1)	3 (4)	0.195
Complicated disease (%)	0 (0)	4 (3)	6 (7)	0.347
Days of hospital stay (range)	12 (11-14)	13 (12-15)	13 (12-15)	0.714
Convalescent faecal carriage (%)	0/10 (0)	0/98 (0)	3/72 (4)	-
Relapse (%)	0/10 (0)	0/98 (0)	0/72 (0)	-

K-M survival curve of the proportion of patients still febrile following the start of treatment



Factors associated with treatment failure

Variable	Treatment failure	Treatment success	*p* value	OR (95% CI)
Number	19	195	-	-
Age years (IQR)	12 (8-22)	13 (8-20)	0.966	-
Days of illness (IQR)	7 (4-14)	8 (6-10)	0.601	-
Male sex (%)	10 (52.6)	92 (47.2)	0.831	1.24 (0.44-3.51)
Mekong Delta site (%)	17 (89.5)	155 (79.5)	0.379	2.19 (0.49-20.3)
Typhi (%)	19 (100)	190 (97)	1.000	-
Paratyphi A (%)	0 (0)	5 (3)	-	-
MDR (%)	15 (78.9)	122 (62.6)	0.242	2.24 (0.68-9.61)
Ciprofloxacin intermediate (%)	17 (89.5)	167 (85.6)	1.00	1.43 (0.31-13.4)
Azithromycin MIC >8 µg/mL (%)	9 (47.4)	76 (39.0)	0.640	1.41 (0.50-3.97)
Duration of azithromycin <7 days (%)	2 (10.5)	40 (20.5)	0.379	0.46 (0.05-2.06)
Dose of azithromycin 10mg/kg (%)	5 (26.3)	40 (20.5)	0.559	1.38 (0.37-4.37)

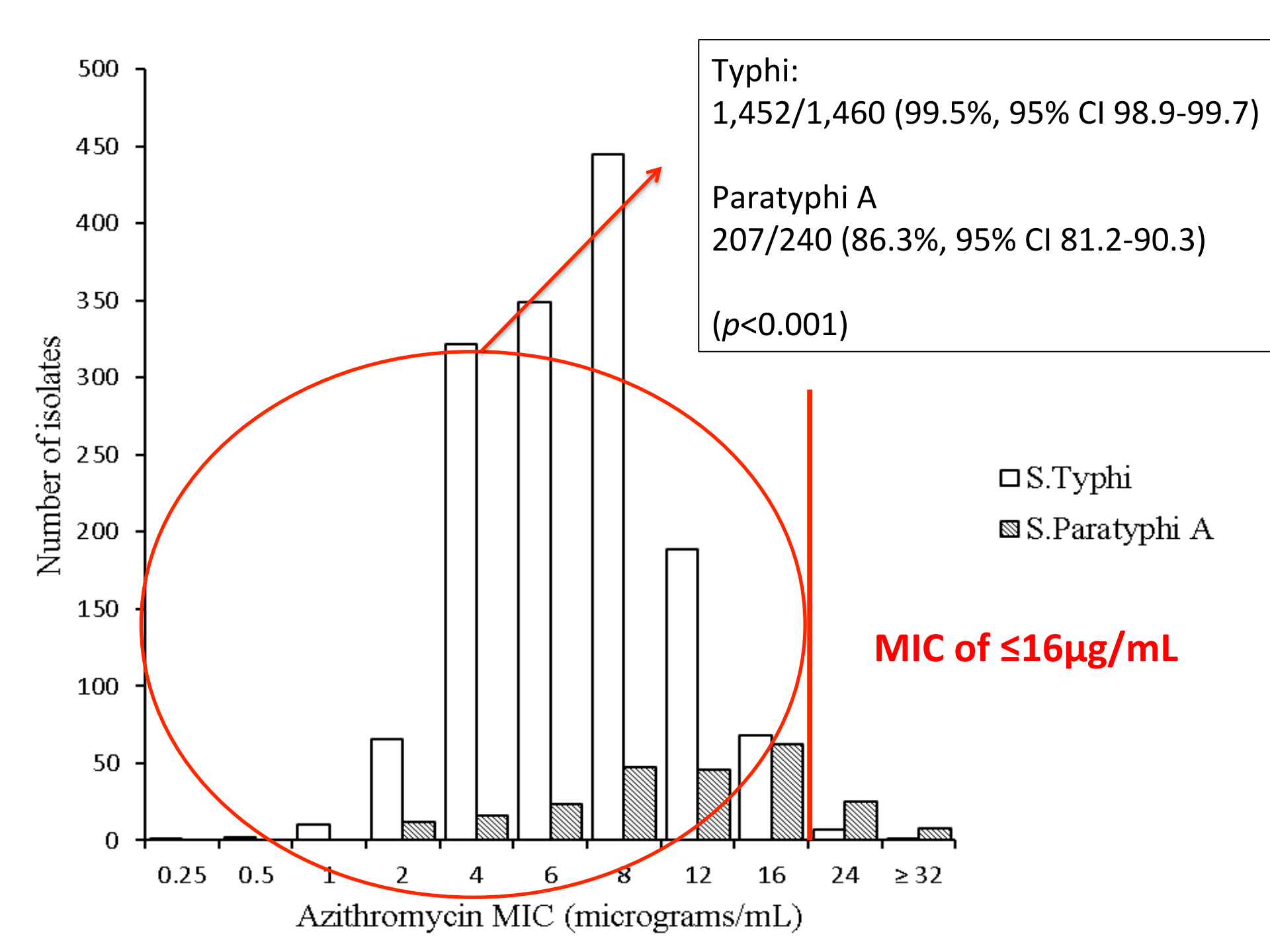
Azithromycin MIC and zone sizes

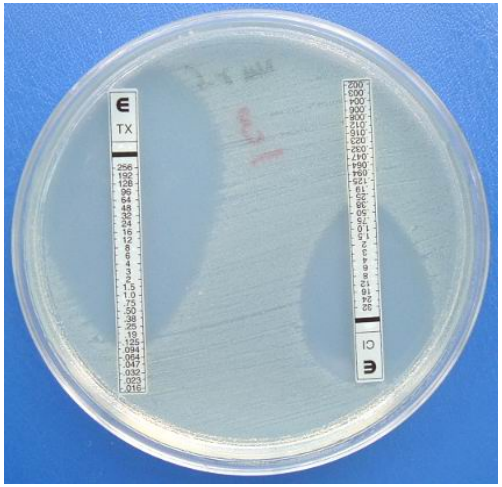
1700 strains:

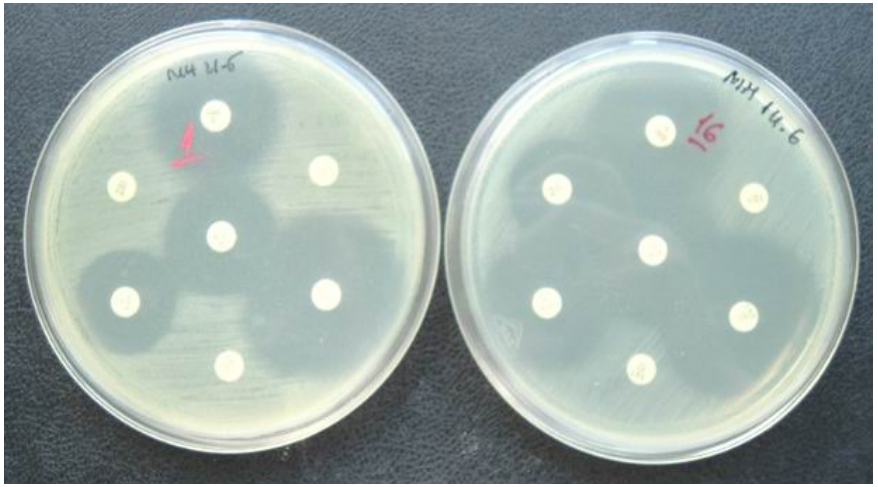
1460 *S.enterica* Typhi

240 *S.enterica* Paratyphi A

Country	1995-2001	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
Bangladesh										32	32
Cambodia					19	28	14	36	50	96	243
India					112	87	106				305
Laos		26	16	35	21	36	33	42	18	6	234
Nepal			160	176	121	76	10	5			548
Thailand					22	20	2	1			45
Vietnam	167	89	38								294
Total											1700

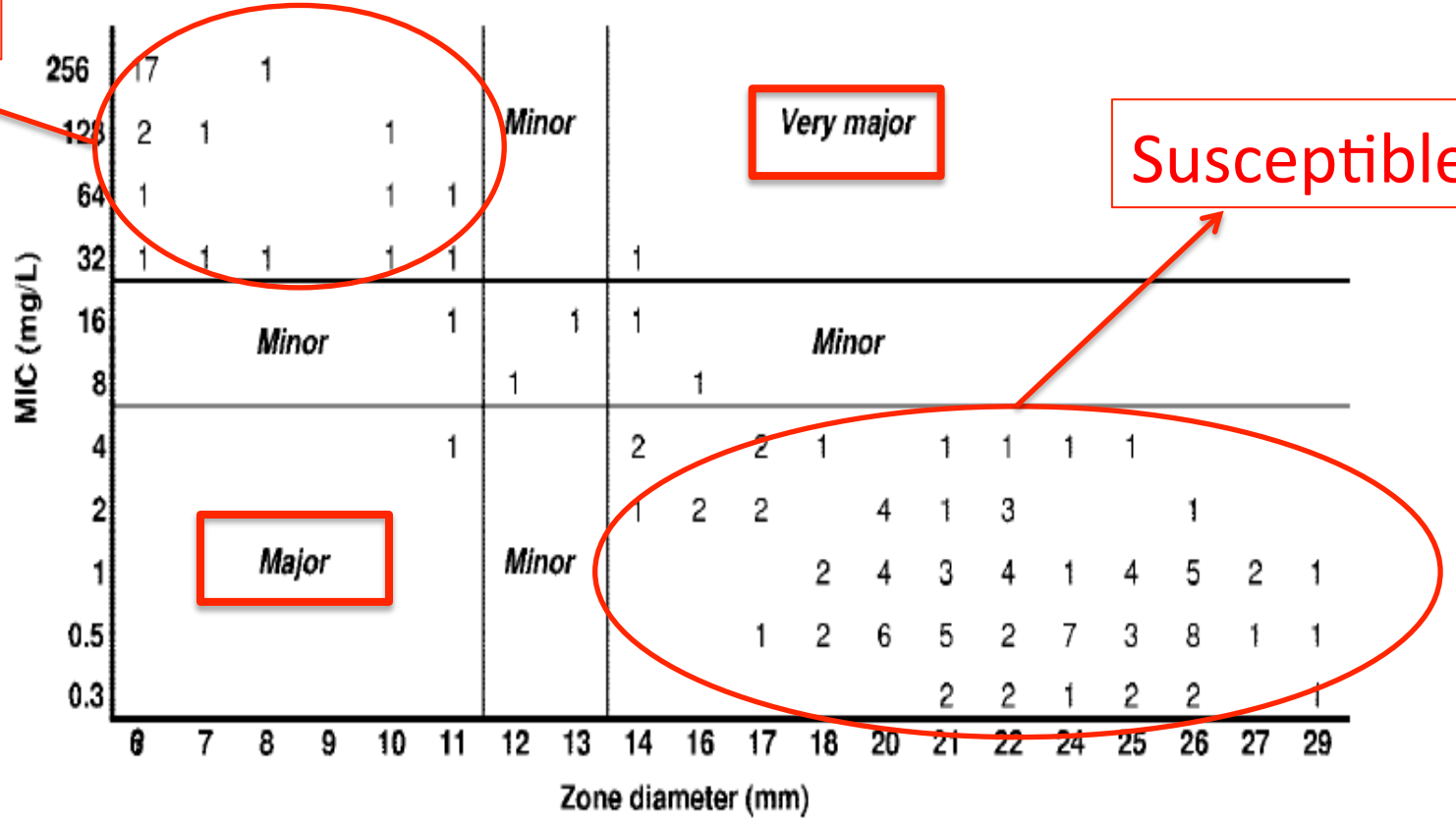

Typhi:
1,452/1,460 (99.5%, 95% CI 98.9-99.7)
Paratyphi A
207/240 (86.3%, 95% CI 81.2-90.3)
(p<0.001)
MIC of ≤16µg/mL
S.Typhi
S.Paratyphi A
Number of isolates
500
450
400
350
300
250
200
150
100
50
0
0.25
0.5
1
2
4
6
8
12
16
24
≥ 32
Azithromycin MIC (micrograms/mL)



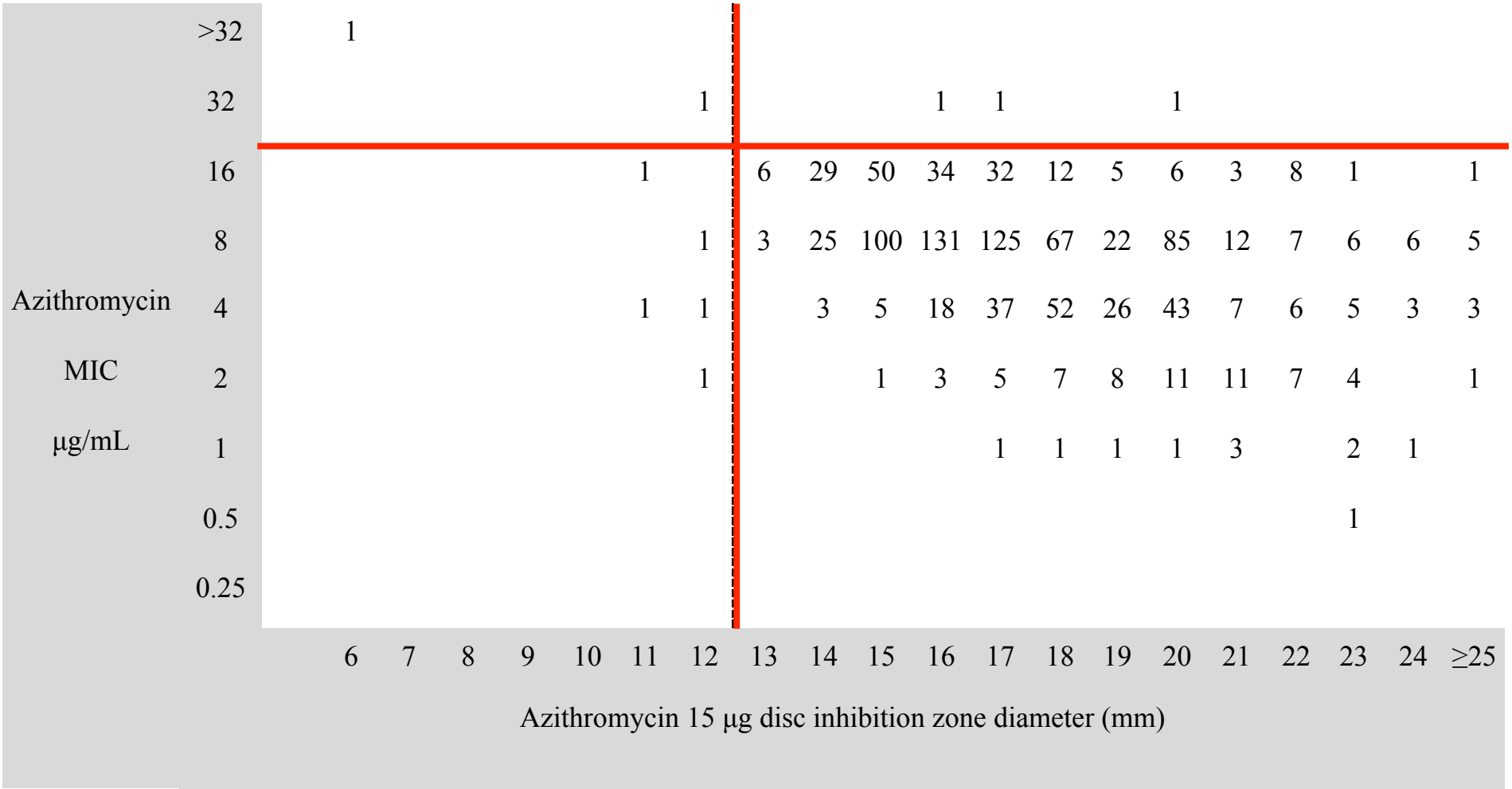


Resistant

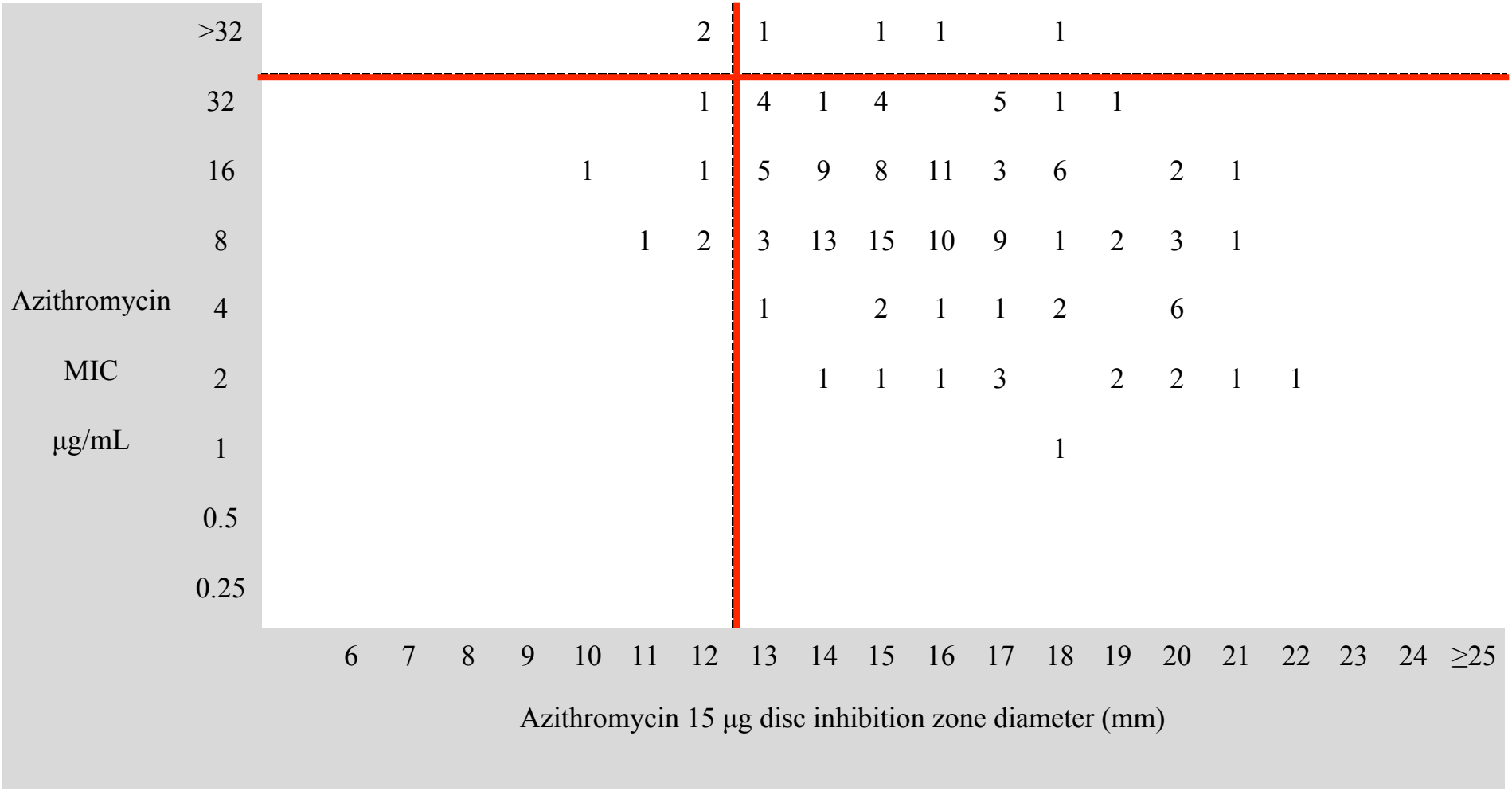
Susceptible



Results – *S.enterica* Typhi



Results – *S.enterica* Paratyphi A



Results – 13mm disc breakpoint

Organism	MIC range[a]	No. of isolates	Number of discrepancies (discrepancy rate [%])	
			Very major	Major
Typhi (Breakpoint	≥ R + 1	1	0	NA
susceptible ≤16	R + S	191	3 (1.6)	1 (0.5)
µg/mL)	≤ S + 1	870	NA	4 (0.5)
	Total	1062	3 (0.3)	5 (0.5)
Paratyphi A	≥ R + 1	0	0	NA
(Breakpoint	R + S	23	4 (17.4)	1 (4.3)
susceptible ≤32	≤ S + 1	133	NA	5 (3.8)
µg/mL)	Total	156	4 (2.6)	6 (3.8)

Conclusion

Our data support the proposition that an azithromycin MIC of ≤ 16 µg/mL defines a wild–type population of Typhi isolates

An azithromycin MIC of ≤16µg/mL Typhi isolates was associated with a favourable response to azithromycin treatment in uncomplicated enteric fever

Tentative MIC and disc susceptibility breakpoints proposed by CLSI January 2015

Limitations

- Difficult to read disc zone size end-point
- Response to treatment in Typhi with an azithromycin MIC>16 µg/mL
- Determine MIC and disk breakpoints for Paratyphi A
- Understand the current MIC distribution of strains globally
- Explore the resistance mechanisms in strains with elevated MICs

Acknowledgments

wellcome trust

Oxford-Lee Ka Shing
Global Health Foundation

Indian Council of Medical Research

OUCRU, Vietnam
Tran Vu Thieu Nga
Christiane Dolecek
Ha Vinh
Nguyen Tran Chinh
Le Thi Phuong
Pham van Be Bay
Tran Thi Phi La
Nguyen Ngoc Rang
James I Campbell
Duy Pham Thanh
Tuyen Ha Thanh
Nguyen Van Sach
Tran Tinh Hien
Vo Anh Ho
Guy Thwaites
Jeremy J Farrar
Stephen Baker

OUCRU, Nepal
Buddha Basnyat
Abhilasha Karkey
Sabina Dongol

COMRU/AHC, Cambodia
Paul Turner
Catrin E Moore
Soeng Sona
Nicholas PJ Day

LOMWRU, Laos
David Dance
Rattanaphone Phetsouvanh
Paul Newton

MORU/CMCH, Bangladesh
Rapeephan R Maude
Aniruddha Ghose
Arjen Dondorp
Mohamed Abul Faiz

Safdarjung Hospital, India
Ruchi Gupta
Rajni Gaind
Monorama Deb

oucru

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE