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Original article

Epidemiological cut-off value and antibiotic susceptibility test methods for azithromycin in a collection of multi-country invasive non-typhoidal *Salmonella*

Bieke Tack [1, 2, *], Marie-France Phoba [3, 4], Phe Thong [5], Palpouguini Lompo [6], Charlien Hupko [7], Stefanie Desmet [2, 8], Delphine Martiny [9, 10], Wesley Mattheus [11], Maria Pardos de la Gandara [12], Lisette Mbuyi-Kalonji [3, 4], Laura Kuijpers [13], Benoit Prevost [9], Barbara Barbé [1], Olivier Vandenberg [14, 15, 16], Octavie Lunguya [3, 4], Joaquim Ruiz [17], Jan Jacobs [1, 2], Liselotte Hardy [1]

[1] *Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium*
[2] *Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium*
[3] *Department of Microbiology, National Institute for Biomedical Research, Kinshasa, Democratic Republic of the Congo*
[4] *Department of Microbiology, University Teaching Hospital of Kinshasa, Kinshasa, Democratic Republic of the Congo*
[5] *Sihanouk Hospital Center of Hope, Phnom Penh, Cambodia*
[6] *IRSS/Clinical Research Unit of Nanoro, Nanoro, Burkina Faso*
[7] *Faculty of Medicine, KU Leuven, Leuven, Belgium*
[8] *Clinical Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium*
[9] *Department of Microbiology, Laboratoire des Hôpitaux Universitaires de Bruxelles, Universitaire Laboratorium Brussel (LHUB-ULB), Bruxelles, Belgium*
[10] *Faculté de Médecine et Pharmacie, Université de Mons (UMONS), Mons, Belgium*
[11] *Division of Human Bacterial Diseases, Sciensano, Uccle, Belgium*
[12] *Institut Pasteur, Université Paris Cité, Unité des Bactéries Pathogènes Entériques, Centre National de Référence des Escherichia coli, Shigella et Salmonella, Paris, France*
[13] *Department of Infectious Diseases, Leiden University Medical Center, Leiden, the Netherlands*
[14] *Center for Environmental Health and Occupational Health, School of Public Health, Université Libre de Bruxelles, Brussels, Belgium*
[15] *Innovation and Business Development Unit, Laboratoire des Hôpitaux Universitaires de Bruxelles, Universitaire Laboratorium Brussel (LHUB-ULB), Bruxelles, Belgium*
[16] *Division of Infection and Immunity, Faculty of Medical Sciences, University College London, London, United Kingdom*
[17] *Grupo de Investigación en Dinámicas y Epidemiología de la Resistencia a Antimicrobianos - "One Health," Universidad Cientifica del Sur, Lima, Peru*

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ABSTRACT

Objective: Azithromycin is an alternative to treat invasive non-typhoidal *Salmonella* (iNTS) infections. We determined its epidemiological cut-off (ECOFF) and compared azithromycin susceptibility testing methods for iNTS.
Methods: We used EUCAST ECOFFinder to determine the minimum inhibitory concentrations (MIC; obtained by broth microdilution) ECOFF and corresponding disk zone diameters of 515 iNTS from blood cultures in Democratic Republic of Congo, Burkina Faso, Rwanda, and Cambodia. Transferable resistance mechanisms were determined by polymerase chain reaction. We compared azithromycin susceptibility testing by semi-automated broth microdilution (customized Sensititre panel; reference), agar dilution, gradient tests (bioMérieux, Liofilchem, HiMedia; read at 80% (MIC80%) and 100% inhibition (MIC100%)), and disk diffusion (Rosco, Oxoid, BD, Liofilchem) for 161 wild- and 198 non–wild-type iNTS.
Results: Azithromycin MIC ECOFF was 16 mg/L corresponding to a 12 mm zone diameter; *mphA* was detected in 192/197 non–wild- and 0/47 wild-type iNTS. Categorical agreement was excellent (≥98%) for all methods. Essential agreement was very good for agar dilution (>90%) but moderate for gradient tests (MIC80%: 52% to 71% and MIC100%: 72% to 91%). Repeatability was good for all methods/brands. Inter-reader agreement was high for broth microdilution and agar dilution (all ≤1 twofold dilution difference) and disk diffusion (>96% ≤3 mm difference) but lower for gradient tests (MIC80% & MIC100%: 83% to 94% ≤1 twofold dilution difference).

* Corresponding author: Bieke Tack, Nationalestraat 155, 2000 Antwerp, Belgium.
E-mail address: btack@itg.be (B. Tack).

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Discussion: Azithromycin ECOFF of iNTS was 16 mg/L, i.e. equal to *Salmonella* Typhi. Disk diffusion is an accurate, precise, and user-friendly alternative for agar dilution and broth microdilution. Reading gradient tests at 100% instead of 80% inhibition improved accuracy and precision. **Bieke Tack, Clin Microbiol Infect 2022;28:1615**

Introduction

Invasive non-typhoidal *Salmonella* infections (iNTS) are a primary cause of bloodstream infections in low-resource settings (LRS). The global burden has been estimated at 535,000 iNTS cases and 77,500 deaths per year [1]. In sub-Saharan Africa, most iNTS are multidrug resistant, and nonsusceptibility to third generation cephalosporins and fluoroquinolones has emerged [2].

In this context [2,3], azithromycin is an important candidate for oral iNTS treatment [2]. Azithromycin is effective to treat typhoid fever [4] and has, despite missing clinical efficacy data, been recommended for (switch to) oral iNTS treatment [2].

Nevertheless, there are no international guidelines on azithromycin antibiotic susceptibility testing (AST) and interpretation for iNTS [5,6]. For *Salmonella* Typhi, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) recommend interpreting azithromycin AST using an epidemiological cut-off (ECOFF) [5,6]. This ECOFF differentiates the minimum inhibitory concentration (MIC) or zone diameter distribution of wild-type *Salmonella* Typhi from *Salmonella* Typhi that acquired an azithromycin resistance mechanism (non–wild type) [7].

In the present study, we determined the azithromycin MIC ECOFF and correlated zone diameter in iNTS isolates from bloodstream infections and confirmed this by molecular resistance mechanisms detection. Next, because MIC reference methods, i.e. manual broth microdilution (BMD) and agar dilution, are challenging because of logistical, technical, and human resource–related constraints in LRS [8], we compared the performance of more user-friendly AST.

Methods

Selection of iNTS isolates and study design

Isolates were retrieved from previous hospital-based blood culture surveillance studies in the Democratic Republic of Congo (DR Congo), Rwanda, Burkina Faso, and Cambodia (Table 1), which

Table 1
Overview of selected iNTS isolates for the determination of the azithromycin ECOFF, for comparison of different azithromycin AST methods, and for PCR to detect molecular azithromycin resistance mechanisms

	Origin	Period	N total	Serotype	Non–wild-type iNTS (based on broth microdilution)	Laboratory work-up
ECOFF n = 515	DR Congo - HGR Kisantu [1]	2017	279	167 Typhimurium 110 Enteritidis 2 Other serotypes	17 Typhimurium 1 Abony	Institute of Tropical Medicine Antwerp (Belgium)
	DR Congo - CH Lwiro [2]	2005–2008	54	54 Typhimurium	None	Sciensano, Brussels (Belgium)
	Rwanda - CH Kigali [2]	1984	120	110 Typhimurium 10 Enteritidis	None	Université Libre de Bruxelles (Belgium)
	Burkina Faso - CRUN Nanoro	2016– 2017	26	12 Typhimurium 13 Enteritidis 1 Other serotypes	None	University Hospitals Leuven (Belgium)
	Cambodia - SHCH Phnom Penh	2007– 2011	36	23 Choleraesuis 7 Enteritidis 4 Typhimurium 2 Other serotypes	17 Choleraesuis	Institut Pasteur, Paris (France)
PCR n = 243	DR Congo surveillance network [1,3]	2007– 2017	206	198 Typhimurium 7 Enteritidis 1 Abony	175 Typhimurium 1 Abony	Universidad Científica del Sur, Lima (Peru)
	Cambodia - SHCH Phnom Penh	2007– 2011 2016– 2018	37	24 Choleraesuis 7 Enteritidis 4 Typhimurium 2 Other serotypes	17 Choleraesuis	Universidad Científica del Sur, Lima (Peru)
Comparison AST methods n = 358	DR Congo surveillance network [1,3]	2007– 2017	311	275 Typhimurium 35 Enteritidis 1 Abony	175 Typhimurium 1 Abony	Institute of Tropical Medicine, Antwerp (Belgium)
	Cambodia - SHCH Phnom Penh	2007– 2011 2016– 2018	47	25 Choleraesuis 14 Enteritidis 5 Typhimurium 3 Other serotypes	17 Choleraesuis	Institute of Tropical Medicine, Antwerp (Belgium)

All iNTS were isolated from blood cultures, sampled as part of hospital-based blood culture surveillance studies. Blood culture surveillance studies were organized with the local partner, in collaboration with Institut National de Recherche Biomédicale [1] or by Centre Hospitalier Universitaire Saint-Pierre Université Libre de Bruxelles [2]. Except for the selection of iNTS from DR Congo for AST comparison, all collections included all iNTS from that origin and period, see methods [3].
AST, antibiotic susceptibility testing; CH, Centre Hospitalier; CRUN, Clinical Research Unit of Nanoro; ECOFF, epidemiological cut-off; HGR, Hôpital Général de Référence; iNTS, invasive non-Typhi *Salmonella*; SHCH, Sihanouk Hospital Centre of Hope.

were identified, serotyped, and biobanked at the Institute of Tropical Medicine (Antwerp, Belgium) [3,9–11].

The ECOFF was calculated with EUCAST ECOFFinder to differentiate wild-from non–wild-type azithromycin distribution based on MIC values of five iNTS collections determined by Sensititre BMD [7,12]. Disk diffusion testing was performed for MIC-zone diameter correlation.

Presence of transferable molecular resistance mechanisms was assessed for all iNTS with MIC >16 mg/L and randomly selected iNTS with MIC ≤16 mg/L (agar dilution MIC: see next paragraph).

We performed comparative azithromycin AST for all Cambodian and a selection of DR Congo iNTS isolates. We selected based on archived azithromycin MIC gradient test results (bioMérieux, Marcy-l'Étoile, France) read at 80% inhibition and included all iNTS with MIC >16 mg/L and a subset with MIC ≤16 mg/L representative for the azithromycin wild-type MIC distribution. We compared agar dilution, gradient, and disk diffusion tests with semi-automated BMD using customized Sensititre plates.

Epidemiological cut-off

MIC and zone diameter distribution and ECOFF calculation

Isolates, stored at −80°C, were inoculated on Columbia agar with 5% sheep blood (blood agar; Becton Dickinson (BD), Franklin Lakes, NJ) and subcultured on Tryptic Soy Agar (Difco TSA; BD) or blood agar for transport to other laboratories.

MIC values were determined with the semi-automated Sensititre AST System (Sensititre Nephelometer, AIM automated inoculator & Vizion digital MIC viewing system; Thermo Fisher, Waltham, MA) with customized, single lot dry Sensititre plates (serial twofold azithromycin dilutions: 0.125 to 512 mg/L; Table S1). For disk diffusion, we used 15 μg azithromycin disks from different brands (Oxoid/Thermo Fisher; BD; Rosco, Taastrup, Denmark; Liofilchem, Abruzzi, Italy) and commercially prepared Mueller-Hinton agar plates (BD).

For each collection, BMD MIC values were determined in a different laboratory (Table 1) according to EUCAST and CLSI guidelines [13–15]. Disk diffusion was performed at the Institute of Tropical Medicine according to EUCAST and CLSI guidelines [16,17], except for the Burkina Faso collection (done at Leuven University Hospital).

Data were aggregated after re-weighting (each distribution received weight = 1 to avoid numerical dominance of large collections) before entry in EUCAST ECOFFinder 2.1 [12]. The ECOFF was visually and numerically (97.5% to 99.9% ECOFF) determined, as described by EUCAST [7]. Zone diameters were plotted against azithromycin MIC to visually assess correlation between MIC and zone diameters.

According to the latest EUCAST BMD reading instructions, pinpoint growth should be ignored for trailing azithromycin endpoints in Gram negatives [18]. Since this change in reading instructions occurred during the study, all MIC were reread *post-hoc* using Sensititre photos and the ECOFF was recalculated (Fig. S3).

Transferable azithromycin resistance mechanism detection

The presence of *erm(A)*, *ere(B)*, *erm(B)*, *erm(C)*, *ere(A)*, *mph(A)*, *mph(B)*, *msr(A)*, *msr(D)*, *mef(A)*, and *mef(B)* genes was determined by polymerase chain reaction (primers and conditions previously described [19,20]). To detect *cfr*, the primers cfr-F (5' - TGTGCTACAGGCGACATTGAT - 3′) and cfr-R (5' - CAAATAC TTTACGGTTGGCTAGAG - 3′) were adapted from Wang et al. [21] and amplified at the following temperatures: 95°C × 3 min, 30 x (94°C × 1 min, 55°C × 1 min, 72°C × 1 min), 72°C × 5 min. Amplified products were visualised in 1.5% agarose gel stained with Sybr Safe (Invitrogen, Carlsbad, CA).

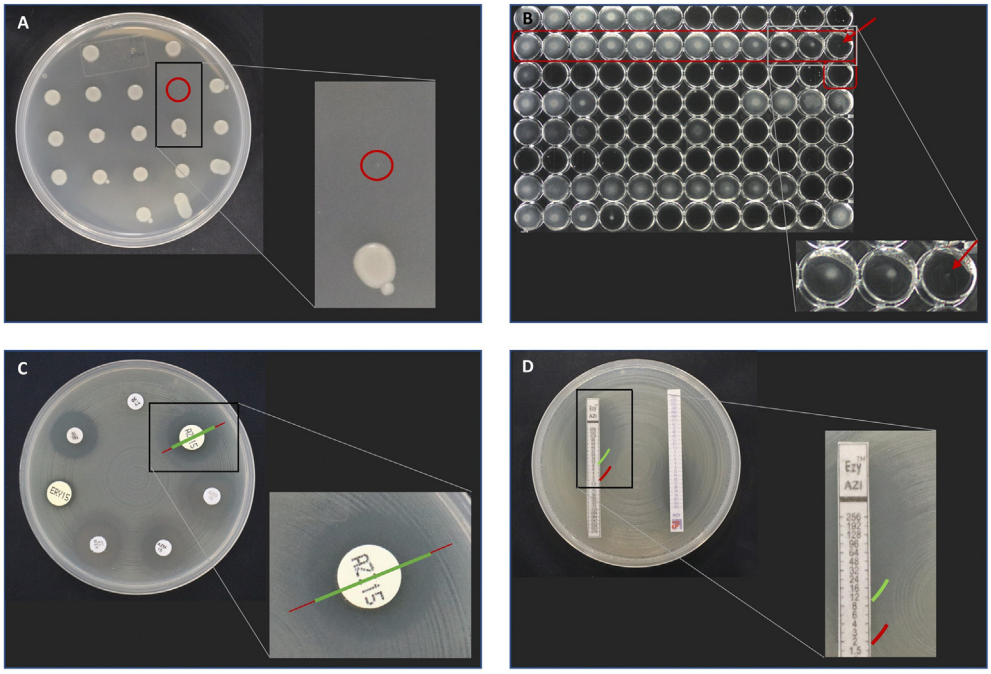

Fig. 1. Reading observations for each azithromycin susceptibility testing method. Panel A & B: Trailing endpoints were frequently observed in agar dilution and semi-automated Sensititre broth microdilution, respectively. MIC was read at complete growth inhibition. Panel C: Double inhibition zones were observed during disk diffusion testing. The inner diameter (green line) was read, according to EUCAST recommendations. Panel D: For gradient testing, the manufacturers recommend reading the Minimum Inhibitory Concentration at 80% inhibition and ignore the haze (red curved line). Results showed that precision and accuracy improved if read at 100% inhibition (green curved line).

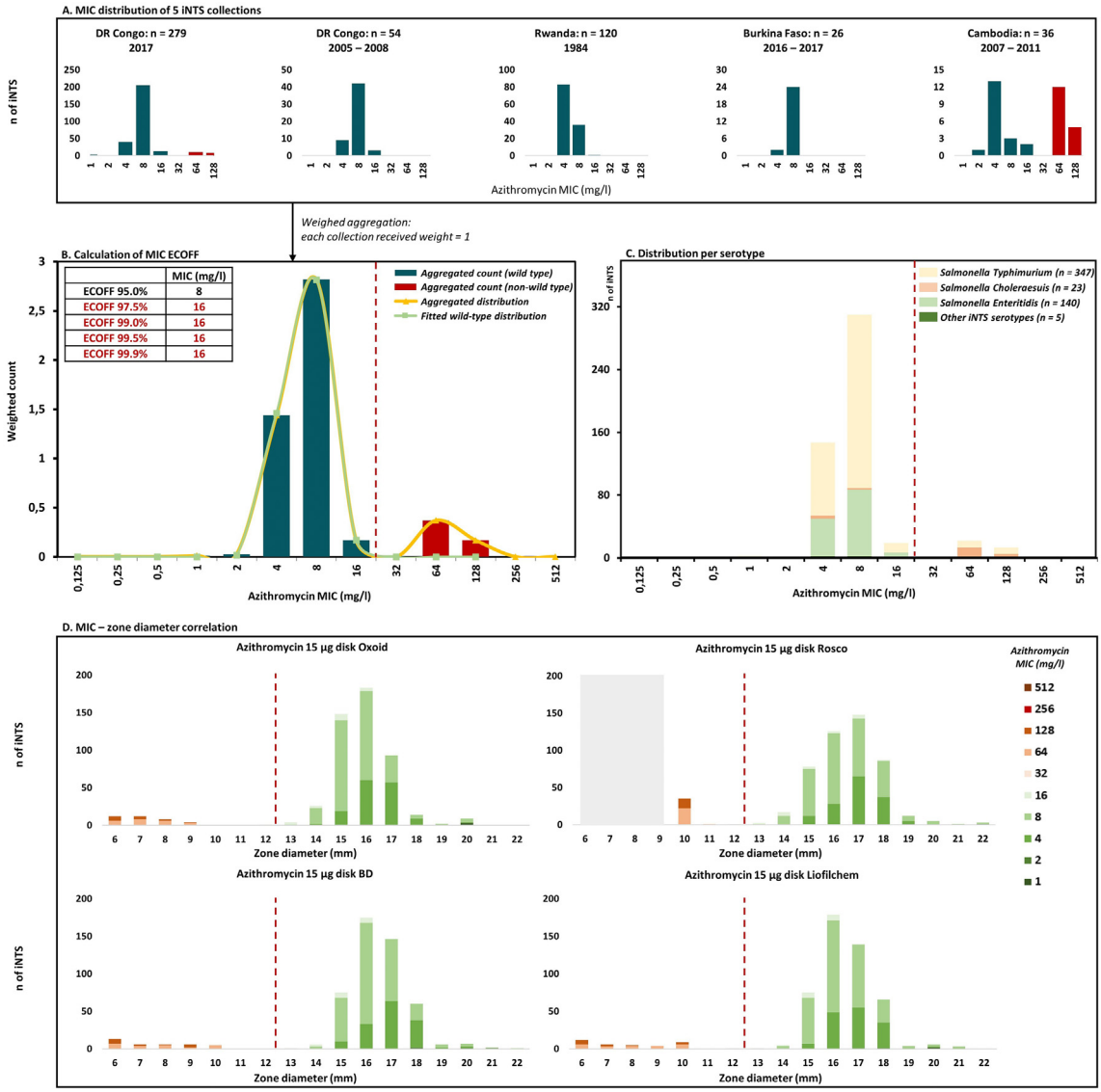

Fig. 2. Azithromycin epidemiological cut-off (ECOFF) in invasive non-typhoidal *Salmonella* (iNTS) isolates (n = 515) from five different blood culture surveillance collections. Panel A: Distribution of azithromycin Minimum Inhibitory Concentration (MIC) obtained by broth microdilution in the 5 iNTS collections: green and red bars indicate wild type and non-wild type iNTS respectively, based on the azithromycin MIC ECOFF of 16 mg/l, calculated with ECOFFinder 2.1 and shown in Panel B. Panel C: Aggregated azithromycin MIC distribution per serotype. Panel D: MIC – zone diameter correlation for 4 different brands of azithromycin 15 µg disks.

Comparative azithromycin AST

Isolates, stored at −80°C, were subcultured on blood agar for inoculation of agar dilution, gradient testing and disk diffusion and stored on TSA for BMD at a later stage.

Per iNTS isolate batch, Mueller-Hinton plates for agar dilution, gradient tests and disk diffusion were prepared using one lot of media (Thermo Fisher) and azithromycin dihydrate (for agar dilution only; Sigma-Aldrich, St. Louis, MO). For BMD, semi-automated Sensititre AST System with customized, dry Sensititre plates (Table S1) was used. Agar dilution and BMD (serial twofold dilutions: 0.125 to 512 mg/L) and azithromycin 15 µg disk diffusion (Oxoid; BD; Rosco; Liofilchem) were performed according to EUCAST and CLSI guidelines [13–17]. Gradient tests (bioMérieux; HiMedia, Mumbai, India; Liofilchem) were performed according to manufacturers' instructions.

Reading was done by two independents observers and a third observer if MIC values differed >1 twofold dilution or zone diameters >2 mm between the two observers. Per BMD batch, mirror reading was compared with digital viewing for *Staphylococcus aureus* ATCC 29213 and one iNTS isolate. According to

Table 2
Interreader agreement of azithromycin susceptibility test methods for iNTS

Minimum inhibitory concentration (n = 359)	% of iNTS within 1 twofold dilution from mode
Semi-automated Sensititre broth microdilution	100%
Agar dilution	100%
Gradient tests MIC80% (all brands)	83.0%–89.7%
Gradient tests MIC100% (all brands)	88.9%–93.9%
Inhibition zone diameter (n = 359)	% of iNTS within 3 mm from mode
Disk diffusion (all brands)	96.4 – 99.7%

MIC80% & MIC100%, minimum inhibitory concentration read at 80% and 100% inhibition.
iNTS, invasive non-Typhi *Salmonella*.

manufacturers' instructions, gradient test azithromycin MIC should be read at 80% inhibition because of its bacteriostatic character but, to detect reading issues, 100% inhibition was also read (Fig. 1). As recommended by EUCAST, inner inhibition zones were read for disk diffusion [22]. Agar dilution and BMD were read at complete growth inhibition, i.e. trailing endpoints presenting as pinpoint or faint growth were considered as growth (Fig. 1) [14–16]. BMD was not reread according to the revised EUCAST instructions [18].

Precision

For each new iNTS isolates batch tested, *S. aureus* ATCC 29213 and 25923 were used for quality control of azithromycin MIC and disk diffusion testing respectively (Table S2) [5,6]. Repeatability of each method and brand was tested with ATCC 29213, one wild-type and one non–wild-type iNTS. The three isolates were suspended in triplicate to obtain three 0.5 McFarland solutions (verified with densitometer (Biosan, Riga, Latvia)), which were in turn used to inoculate three Mueller-Hinton agars/broths. For BMD, two Sensititre plates were inoculated per Mueller-Hinton broth. The ISO-criteria for reproducibility were adopted to interpret repeatability and interreader agreement [13].

Accuracy

Essential and categorical agreement and (very) major discrepancies compared to BMD were calculated and interpreted according to ISO standards [13]. To evaluate categorical agreement, the ECOFF calculated in the present study and its corresponding zone diameter were used. Bland-Altman analysis and intraclass correlation coefficients calculation (calculated for single log2-transformed values with a two-way model for absolute agreement) were performed in R version 3.6.1 (packages "BlandAltmanLeh" and "irr").

Results

Azithromycin epidemiological cut-off for iNTS

Azithromycin MIC ECOFF and correlating zone diameter

All collections had similar wild-type azithromycin MIC distributions (Fig. 2A). The azithromycin MIC ECOFF obtained by the ECOFFinder was 16 mg/L (ECOFF 97.5% to 99%; Fig. 2B). From 515 iNTS, 35 iNTS had a MIC >16 mg/L and were considered non–wild type, i.e. 17 *Salmonella enterica* serovar Typhimurium (*Salmonella* Typhimurium), 1 *Salmonella* enterica serovar Abony (*Salmonella* Abony) from DR Congo and 17 *Salmonella enterica* serovar Choleraesuis (*Salmonella* Choleraesuis) from Cambodia. The calculated ECOFF did not substantially differ when applying the new EUCAST reading instructions ignoring pinpoint growth in case of trailing endpoints (Fig. S3). The wild-type MIC distribution was similar for all iNTS serotypes (Fig. 2C). Wild-type iNTS had a zone diameter of >12 mm in ≥99.7% (479/480; Fig. 2D): there was one wild-type iNTS (MIC = 16 mg/L) with a 12 mm zone diameter with disks from Oxoid, Rosco and Liofilchem, but 13 mm with BD disks.

Transferable azithromycin resistance mechanisms

Presence of *mph(A)* was confirmed in 98.9% (173/175) *Salmonella* Typhimurium, 1/1 *Salmonella* Abony and 17/17 *Salmonella* Choleraesuis with non–wild-type MIC based on agar dilution (>16 mg/L). Among these 173 isolates, co-presence of *ere(B)* was demonstrated in one *Salmonella* Typhimurium (BMD MIC: 64 mg/L). Both non–wild-type *Salmonella* Typhimurium without *mph(A)* had BMD MIC values of 64 mg/L. Wild-type iNTS ($n = 50$) did not harbour *mph(A)*, but *erm(B)* was found in one *Salmonella* Choleraesuis with a BMD MIC value of 8 mg/L. None of the other mechanisms tested was found.

Comparison of azithromycin AST methods and brands

Precision

For all methods and brands, MIC and zone diameters of both ATCC strains tested were within quality control ranges (Table S2). For all methods and brands, repeatability testing revealed limited variability, i.e. per method, difference was maximum one twofold dilution or 3 mm from their respective mode (Table S3).

Interreader agreement was excellent for BMD and agar dilution and very good for disk diffusion. For gradient tests, interreader agreement improved when MIC was read at 100% inhibition instead of 80% inhibition (Table 2; Table S4). Semi-automated digital reading of Sensititre plates corresponded well to mirror reading: identical MIC value in 5/5 ATCC 29213 and 5/6 iNTS with one twofold dilution difference for the remaining isolate. Trailing endpoints were observed for 87 (24.2%) and 113 (31.5%) iNTS in BMD and agar dilution, respectively (Fig. 1).

Accuracy

Compared to semi-automated Sensititre BMD, agar dilution complied to all test accuracy ISO-criteria: essential agreement ≥90%, categorical agreement ≥90%, and ≤3% (very) major discrepancies (Fig. 3). Bland-Altman analysis demonstrated a median difference of one twofold dilution between BMD and agar dilution, with lower MIC by BMD (Fig. 3). For all gradient tests read at 80% inhibition, essential agreement was insufficient (Liofilchem: 52.0%, bioMérieux: 57.3%, HiMedia: 71.2%) caused by systematically underestimated MIC of wild-type iNTS (Fig. 3; Figs. S1 and S2). Reading at 100% inhibition partially resolved underestimation and improved essential agreement to 71.8%, 88.6%, and 90.8% for Liofilchem, bioMérieux, and HiMedia, respectively (Fig. 3; Figs. S1 and S2). Categorical agreement was sufficient for gradient tests when read at 80% and 100% inhibition (99.2% to 100% and 99.2% to 99.4%, respectively) and few (very) major discrepancies occurred (Figs. 3 and Figs. S2). For disk diffusion (all brands), categorical agreement was good and few (very) major discrepancies occurred (Fig. 4).

Discussion

In the present study, the azithromycin MIC ECOFF of iNTS was set at 16 mg/L, almost perfectly corresponding to a zone diameter of 12 mm and the presence of macrolide resistance phosphotransferase A (*mph(A)*). In addition, MIC determined by semi-automated Sensititre BMD and agar dilution were precise and corresponded well. Gradient testing had suboptimal precision and accuracy for all brands when read at 80% inhibition, as recommended by the manufacturers, but this improved when read at 100% inhibition. Disk diffusion was precise and accurately discriminated between wild- and non–wild-type iNTS.

The calculated azithromycin MIC ECOFF for iNTS is identical to the one recommended by CLSI and EUCAST for *Salmonella* Typhi [5,6] and the MIC and zone diameter ECOFF for *Salmonella enterica* recommended by EUCAST [7]. However, CLSI (wild type: ≥13 mm) and EUCAST (wild type: ≥12 mm) differ in disk diffusion ECOFF for *Salmonella* Typhi [5,6]. Because of few isolates with a 11 to 13 mm zone diameter to correlate with MIC values, we preferred to harmonize the zone diameter iNTS ECOFF with the most conservative *Salmonella* Typhi ECOFF, i.e. CLSI. Finally, our data correspond with previous data from invasive and noninvasive human, animal, and food non-typhoidal *Salmonella* isolates, in which non–wild-type *Salmonella* had azithromycin MIC values > 16 mg/L [23–27] and presented *mph(A)* [24–26] or a mutation in 50S ribosomal

protein L4 (*rplD*) [23]. The present study was the first to compare different azithromycin AST methods and brands for *Salmonella*. In contrast to a previous EUCAST study [28], we did not find differences in performance between disk brands. Liofilchem gradient tests, however, were less precise and accurate than those from bioMérieux or HiMedia. The perfect gradient test performance for *S. aureus* ATCC29213, but poor performance for iNTS, suggests insufficient calibration for *Salmonella*.

This study is limited by the absence of reproducibility testing but stands out in comprehensiveness. A large, multi-country collection of iNTS was tested with all culture-based azithromycin AST methods and all gradient test/disk brands available on the European Union market. For comparative testing, nonmethod-related variation was limited by use of single lot Mueller-Hinton agar and Sensititre plates and a fixed laboratory technician team. Reading errors were prevented by double

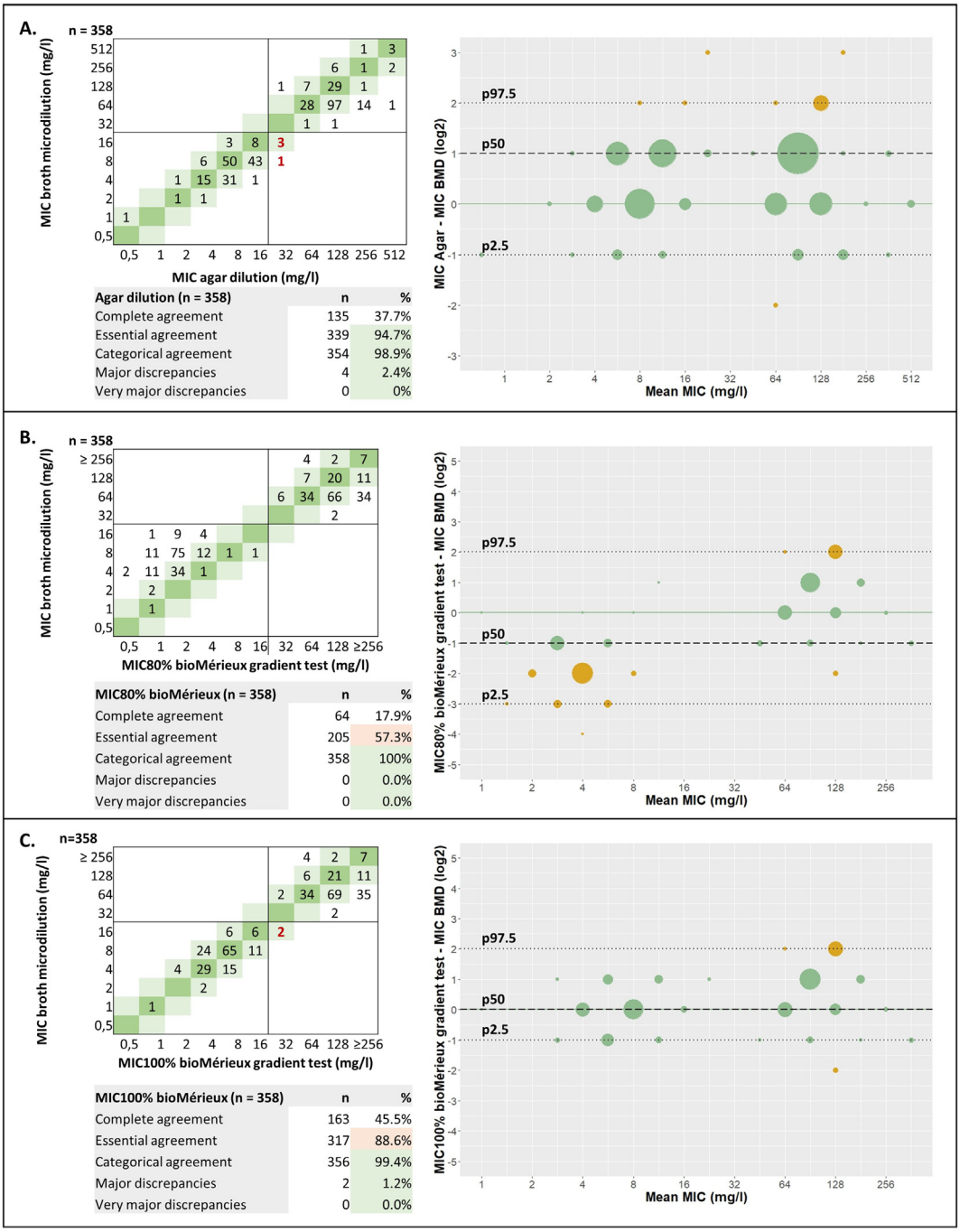

Fig. 3. Essential and categorical agreement between MICs determined by agar dilution (panel A) and bioMérieux gradient tests read at 80% and 100% inhibition (panel B and C respectively) versus semi-automated customized Sensititre broth microdilution (BMD; reference method). To determine agreement, the epidemiological cut-off calculated in the present study was used to discriminate wild type and non-wild type iNTS. Legend Bland-Altman plots: The dashed line represents the median log2 difference with percentiles 2.5 and 97.5 displayed as dotted lines. The ideal situation (no difference) is displayed as a full green line. Bubble sizes reflect the number of iNTS isolates, bubble colour reflects the difference between MIC measured by agar dilution/gradient test and broth microdilution (green: ≤ log2 difference; yellow: > log2 difference).

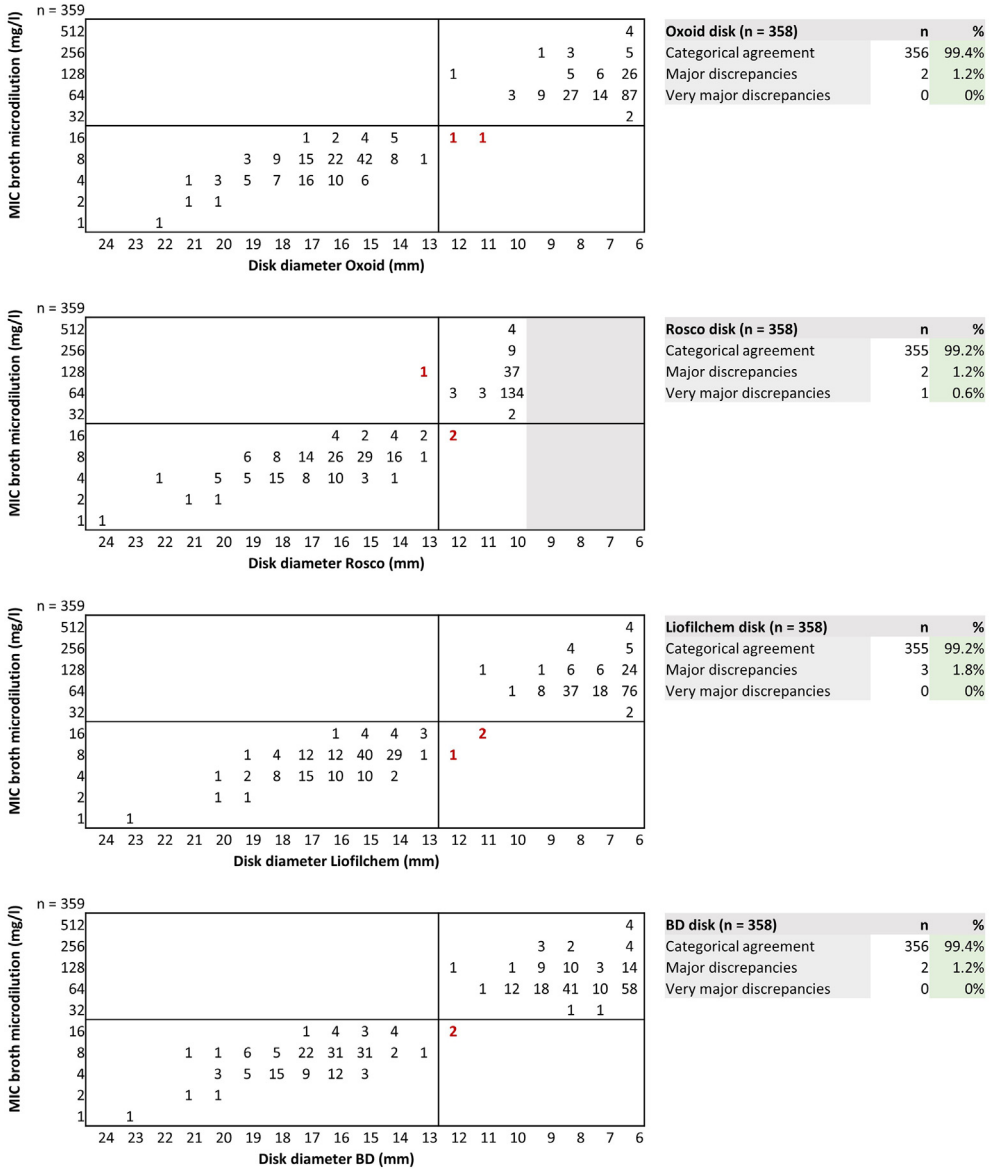

Fig. 4. Categorical agreement between azithromycin susceptibility determined by disk diffusion compared to broth microdilution (reference method). The epidemiological cut-offs calculated in the present study MIC value of 16 mg/l with corresponding zone.

reading, a third observer in case of discrepancies, and mirror-based confirmation of digital reading. However, semi-automated BMD on customized dry Sensititre plates may not be the perfect reference, particularly because EUCAST changed reading BMD instructions during the study course (ignore pinpoint growth for trailing endpoints) [18]. Similarly, for agar dilution, MIC were read at complete growth inhibition as recommended by CLSI [15], while an older EUCAST document recommended ignoring pinpoint/faint growth in agar dilution [29]. For MIC ECOFF determination, each collection was tested in a different laboratory to take into account interlaboratory variation. The narrow wild-type MIC distribution indicated limited variation and, although slightly skewed, clearly distinguished between wild- and non-wild-type iNTS. During disk diffusion for correlation with MIC ECOFF, delineation of inner inhibition zones was fainter than during comparative AST, which complicated reading and was probably due to lower opacity of commercial Mueller Hinton agar plates used in the former tests. Finally, we did not assess all azithromycin resistance mechanisms known in *Salmonella*, e.g. no search for the AcrB efflux pump point mutation seen in Asia [30].

In contrast to intestinal non-typhoidal *Salmonella* infections that generally do not require antibiotic treatment, azithromycin AST is essential to guide antibiotic treatment of iNTS in LRS [2]. Rapid emergence of antimicrobial resistance increases the importance of azithromycin as treatment candidate [2]. However, azithromycin is threatened by its popularity, e.g. in mass drug administration campaigns to reduce childhood mortality or as COVID-19 treatment [31,32]. Awaiting further pharmacokinetic and clinical efficacy studies allowing the establishment of azithromycin breakpoints for iNTS, an ECOFF enables azithromycin surveillance and stewardship [7].

The present study confirmed that the azithromycin ECOFF of *Salmonella* Typhi recommended by CLSI and EUCAST can be expanded to iNTS [5,6]. A universal azithromycin ECOFF for invasive *Salmonella* facilitates implementation in LRS, where serotyping is often unavailable [8]. Easy and reliable discrimination between

wild- and non–wild-type iNTS by disk diffusion further facilitates AST in LRS [8]. For multicentred studies or reference laboratories, semi-automated Sensititre BMD is attractive because of high precision, accuracy, user friendliness, and possibility to use single-lot customized panels.

Transparency declaration

The authors declare no competing interests. This work was funded by the Baillet-Latour fund. BT has a scholarship from Research foundation Flanders (FWO, 1153220N & 1153222N). JJ has received funding from the European Union's Horizon 2020 research and innovation programme under the Vacc-iNTS project, grant agreement No 815439. *Salmonella* isolates were collected in the blood culture surveillance, funded by the Belgian Directorate of Development Cooperation (DGD) through Framework Agreement between the Belgian DGD and the Institute of Tropical Medicine, Belgium. The surveillance activities in Burkina Faso were partially funded by Sysmex Europe GmbH. The full dataset is available at 10.6084/m9.figshare.19016075.

Author contributions

BT conceptualized the study and developed its methodology, contributed to laboratory testing, curated, analysed, and visualised the data, and wrote the manuscript. MFP, PT, PL, LMK, and OL collected the iNTS isolates and reviewed the manuscript. CH participated in laboratory testing, data curation and reviewed the manuscript. SD, DM, BP, WM, and MPDLG participated in laboratory testing and reviewed the manuscript. LK and BB conceptualized the study, organized pilot testing, and reviewed the manuscript. OV shared isolates from a historical collection, participated in laboratory testing, and reviewed the manuscript. JR conceptualized the study, performed the molecular laboratory testing, and reviewed the manuscript. JJ conceptualized and supervised the study and reviewed the manuscript. LH conceptualized and supervised the study, organized and contributed to the laboratory testing, and reviewed the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2022.06.009.

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