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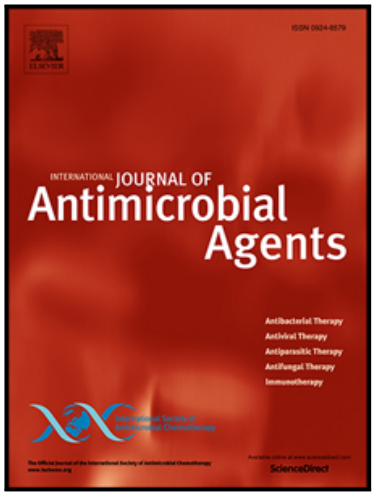

Apramycin susceptibility of multidrug-resistant Gram-negative blood culture isolates in five countries in South-East Asia

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Highlights

- Apramycin demonstrates best-in-class activity against MDR Gram-negative bacilli
- High coverage of pathogens resistant to carbapenems, aminoglycosides, colistin
- Collective data for 470 isolates generated across fives sites in Southeast Asia
- Findings warrant continued development for the treatment of blood stream infections

Apramycin susceptibility of multidrug-resistant Gram-negative blood culture isolates in five countries in South-East Asia

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Abbreviations

3GCR – third-generation cephalosporin resistant

AGR – aminoglycoside resistant

AME – aminoglycoside-modifying enzyme

AMK – amikacin

AMR – antimicrobial resistance

APR – apramycin

BSI – bloodstream infection

CLSI – Clinical and Laboratory Standards Institute

COMRU – Cambodia-Oxford Medical Research Unit

CR – carbapenem resistant

CRE – carbapenem-resistant *Enterobacterales*

CST – colistin

CSTR – colistin resistant

EUCAST – European Committee on Antimicrobial Susceptibility Testing

GEN – gentamicin

GNB – Gram negative bacilli

LMIC – Low- and middle-income countries

LOMWRU – Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit

MDR – multidrug resistant

MIC – minimal inhibitory concentration

NCID – Singapore National Centre for Infectious Diseases

NIHE – Vietnam National Institute of Hygiene and Epidemiology

PKPD – pharmacokinetic pharmacodynamic

PLZ – plazomicin

RMTase – ribosome methyltransferase

SMRU – Shoklo Malaria Research Unit

TOB – tobramycin

Abstract (250 words limit)

Bloodstream infections (BSIs) are a leading cause of sepsis, a life-threatening condition that contributes significantly to the mortality of bacterial infections. Aminoglycoside antibiotics such as gentamicin or amikacin are essential medicines in the treatment of BSIs, but their clinical efficacy is increasingly compromised by antimicrobial resistance. The aminoglycoside apramycin has demonstrated preclinical efficacy against aminoglycoside- and multidrug-resistant (MDR) Gram-negative bacilli (GNB) and is currently in clinical development for the treatment of critical systemic infections.

Here, we collected a panel of 470 MDR GNB isolates from health care facilities in Cambodia, Laos, Singapore, Thailand, and Vietnam for a multi-centre assessment of their antimicrobial susceptibility to apramycin in comparison to other aminoglycosides and colistin by broth microdilution assays.

Apramycin and amikacin MICs ≤ 16 µg/mL were found for 462 (98.3%) and 408 (86.8%) GNB isolates, respectively. Susceptibility to gentamicin and tobramycin (MIC ≤ 4 µg/mL) was significantly lower at 122 (26.0%) and 101 (21.5%) susceptible isolates, respectively. Of note, all carbapenem- and third-generation cephalosporin (3GC) resistant *Enterobacterales*, all *Acinetobacter baumannii*, and all *Pseudomonas aeruginosa* isolates tested in this study appeared to be susceptible to apramycin. Of the 65 colistin-resistant isolates tested, only four (6.2%) had an apramycin MIC > 16 µg/mL.

Apramycin demonstrated best-in-class activity against a panel of GNB isolates with resistances to other aminoglycosides, carbapenems, 3GC, and colistin, warranting continued consideration of apramycin as a drug candidate for the treatment of multidrug-resistant BSIs.

Keywords (3-6)

Bloodstream infection, blood culture isolates, Gram negative, antimicrobial resistance, aminoglycoside, apramycin

1. Introduction

Bacterial bloodstream infections (BSIs) are a leading cause of sepsis [1]. Early diagnosis and effective treatment of BSIs are key in reducing the risk of sepsis, a life-threatening organ dysfunction caused by dysregulation of the host immune response to infection [2]. Sepsis contributes to a large part of global mortality. In 2017, approximately one fifth of all-cause global deaths were due to sepsis, and children under the age of five accounted for 26% of these sepsis-related deaths. Factors affecting the incidence of infections include clean water and sanitation, poverty, food safety, and population density. Good health infrastructure and early and effective infection prevention measures help avert or mitigate the severity of infections and their downstream complications but are often lacking in lower-resource healthcare settings. As a result, the main burden of sepsis mainly affects low- and middle-income countries (LMICs), with a high concentration in Sub-Saharan Africa, South and South-East Asia [2-4]. Alarmingly, the global incidence of sepsis cases caused by multidrug-resistant (MDR) Gram-negative bacteria is on the rise, with children and infants in resource-limited healthcare settings being at particular risk [4, 5].

Empiric treatment guidelines published by the World Health Organization (WHO) recommend the use of an aminoglycoside in combination with a β-lactam antibiotic as first-line treatment against sepsis, and third-generation cephalosporins as second-line therapy. The aminoglycosides gentamicin and amikacin are classified by the WHO as essential medicines with “access” status in its AWaRe classification [6, 7]. They are often a key component in first-line treatment regimens not only in empiric therapy, but also targeted therapy against ESBL-producing and carbapenem-resistant Gram-negative bacteria. Extensive antimicrobial resistance (AMR) has increasingly challenged the empirical treatment approach [4] and led to discussions about optimal therapy in areas of increasing Gram-negative resistance, and treatment adjustments based on the causative agent and its antibiotic susceptibility pattern [8].

The quest for a next generation of aminoglycoside therapeutics not compromised by widespread aminoglycoside resistance or drug safety concerns led to a revitalized interest in the natural product apramycin, a unique octadiose-monosubstituted 2-deoxystreptamine listed by the WHO as a critically important antimicrobial for human medicine [9, 10]. Apramycin circumvents cross-resistance to other aminoglycosides in clinical use by means of a distinct chemical structure that evades enzymatic inactivation by aminoglycoside-modifying enzymes (AMEs) and can still bind and inhibit ribosomes methylated by ribosome-methyltransferases (RMTases), resulting in superior coverage of highly drug-resistant bacterial pathogens [11, 12]. Preclinical evidence has suggested potent in-vivo efficacy of apramycin against both carbapenem- and aminoglycoside-resistant Gram-negative rods and an improved safety profile of apramycin when compared to other aminoglycosides [13-15]. However, its therapeutic potential in various infectious disease indications has yet to be confirmed more specifically for potential target patient populations with a high unmet medical need.

To assess the activity of apramycin in comparison to standard-of-care aminoglycosides and colistin against bacterial blood culture isolates, we performed apramycin susceptibility testing with a panel of 470 MDR Gram-negative bacterial isolates from paediatric and adult patients in South-East Asia.

2. Material and Methods

2.1. Clinical bacterial isolates

We selected a panel of 470 Gram-negative bacilli (GNB) comprising *Escherichia coli*, *Klebsiella pneumoniae, Enterobacter* spp., *Acinetobacter* spp., and *Pseudomonas aeruginosa* (**Table S1**). Bacterial isolates were collected from paediatric and adult BSI patients in Cambodia (Angkor Hospital for Children, Cambodia-Oxford Medical Research Unit (COMRU)), Laos (Mahosot Hospital, Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU)), Thailand (Shoklo Malaria Research Unit (SMRU)), and Vietnam (National Institute of Hygiene and Epidemiology (NIHE), Hanoi, Vietnam). Bacterial isolates contributed by the National Centre for Infectious Diseases (NCID) and Tan Tock Seng Hospital (TTSH) in Singapore included isolates of blood culture and other sample sources. Standard antimicrobial susceptibility testing in accordance with either the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or the Clinical and Laboratory Standards Institute (CLSI) provided for a phenotypic pre-selection of bacterial isolates with a bias towards third-generation cephalosporin resistance (3GCR), carbapenem resistance (CR), colistin resistance (CSTR), aminoglycoside resistance (AGR), or a combination thereof in MDR clinical isolates. Sequential isolates of the same organism from the same patient were not included in this study. Details of EUCAST and CLSI methodologies, interpretative criteria applied, and additional site specifications of relevance with regards to Microbiology Investigation Criteria for Reporting Objectively (MICRO) [16] are summarized and referenced in **Table S2** for each site.

2.2. Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibilities were tested by broth microdilution assays following the CLSI guidelines to assess the activity of apramycin (Sigma, Germany) in comparison to standard aminoglycosides amikacin, gentamicin and tobramycin (European Pharmacopeia reference standards, France), plazomicin (ZEMDRI® medicinal product from the dispensary) and colistin (European Pharmacopeia, France). *E. coli* ATCC 25922 was used as a quality control strain.

2.3. AST interpretation

Interpretative criteria applied in the present study were in accordance with CLSI M100 Performance Standards for Antimicrobial Susceptibility Testing 32nd Edition 2022. Clinical resistant breakpoints for apramycin do not exist. The amikacin breakpoints were tentatively applied as interpretative cut-off values for apramycin in this study, based on previous reports indicating the in-vitro potency and PKPD of apramycin resembles that of amikacin in models using amikacin-susceptible strains [17-19]. For the aminoglycoside plazomicin, the FDA-Identified Susceptibility Test Interpretive Criteria (STIC) for *Enterobacterales* were applied. Interpretative criteria for plazomicin activity against *A. baumannii* and *P. aeruginosa* were not available.

3. Results

3.1. Overall susceptibility profiles

The majority of pathogens in the collected isolate panels belonged to the order of *Enterobacterales* (*n* = 422, 90%), including *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus mirabilis,*

Citrobacter freundii, Serratia liquefaciens, Serratia marcescens, Raoultella terrigena, *Raoultella planticola/ornithinolytica, Morganella morganii, Citrobacter amalonaticus, Leclercia adecarboxylata* and *Kluyvera georgiana*. *Acinetobacter* spp. and *Pseudomonas aeruginosa* were represented with 30 (6%) and 18 (4%) isolates in the panel, respectively (**Table S1**).

The overall susceptibility profiles are shown in **Fig. 1** and summarized in **Table 1**, with further species differentiation within the *Enterobacterales* provided in **Table S3**. *Enterobacterales* isolates were found to be more susceptible to apramycin (MIC_{90} = 8 µg/mL) than to any of the other drugs tested. Although susceptibility to amikacin (91.0% susceptible, MIC_{90} = 16 µg/mL) and plazomicin (83.6% susceptible, MIC_{90} = 8 µg/mL) was still reasonable in comparison to gentamicin and tobramycin (< 30% susceptible, MIC_{90} ≥ 64 µg/mL). Of note, 70 (16.6%) of the 422 *Enterobacterales* isolates studied were found to be resistant to colistin when applying the CLSI cut-off of ≥ 4 µg/mL.

The discrepancy between apramycin and other aminoglycosides was even more pronounced for the *Acinetobacter* spp. and *P. aeruginosa*, with none of the BSI isolates tested being resistant to apramycin or colistin.

3.2. Susceptibility by resistance profiles

Next, we stratified the susceptibility results by phenotypic resistance, because the medical need for novel treatment options concentrates around bacterial pathogens that are resistant to existing second-line or last-resort antibiotics. Susceptibility data for third-generation cephalosporins and carbapenems were available for 324 strains from all sites except Vietnam. **Fig. 2** shows the MIC distributions for individual subsets of 3GCR, CR, CSTR, and AGR isolates.

The MIC distributions for the 282 3GCR isolates and the 84 CR isolates resembled the patterns already observed for the overall susceptibility profiles presented above. All 3GCR and CR *Enterobacterales, Acinetobacter* spp. and *P. aeruginosa* isolates were susceptible to apramycin (**Fig. 2**).

Sixty-two (93.9%) of the 66 colistin-resistant isolates were susceptible to apramycin, compared to 56 (84.8%) colistin-resistant isolates susceptible to amikacin. Gentamicin and tobramycin showed lower coverage of colistin-resistant isolates (**Fig. 2** and **Table S4**).

Of the 60 aminoglycoside-resistant isolates that were resistant to amikacin, gentamicin, tobramycin, and plazomicin, only a single *K. pneumoniae* isolate was also resistant to apramycin, with an apramycin MIC of 64 µg/mL. In comparison, nine of the 60 aminoglycoside-resistant isolates were also resistant to colistin (**Fig. 2** and **Table S5**). Plotting the apramycin MIC against the amikacin MIC of each isolate suggests a near equivalency in antibacterial potency of these two aminoglycosides when targeting aminoglycoside-susceptible isolates, and a nearly full coverage of amikacin-resistant isolates by apramycin (**Fig. S1**).

Since bacterial susceptibility to apramycin was one of our main objectives in the present study, we were also particularly interested in the susceptibility profile of the four isolates found to be less susceptible to apramycin, three *E. coli* and one *K. pneumoniae* with an apramycin MIC > 32 µg/mL.

Interestingly, two of the four isolates retained susceptibility to amikacin only, one isolate to colistin only, and the fourth isolate to amikacin, plazomicin, and colistin (**Table S6**).

4. Discussion

Our findings indicate that apramycin exhibits best-in-class antimicrobial activity against GNB blood culture isolates, because it retains antibacterial coverage of carbapenem-resistant isolates that are frequently also found to be resistant to gentamicin, tobramycin, amikacin, and plazomicin. Amikacin, plazomicin, and colistin showed lower coverage of resistant isolates than apramycin, but higher coverage of *Enterobacterales* isolates than gentamicin and tobramycin. Somewhat surprisingly, amikacin appeared to demonstrate better coverage than plazomicin against the specific *Enterobacterales* panel studied here, which has a specific selection bias for multidrug-resistant phenotypes. For the 470 isolates tested, apramycin showed higher coverage than colistin not only overall, but also in the aminoglycoside-resistant subpopulation. Only four isolates (0.85%) were found to be resistant to apramycin, the lowest rate of all drugs tested in this study.

In South-East Asia, the prevalence of drug resistance varies but can reach up to over 70% of 3GCR *E. coli* and up to over 50% of CR *K. pneumoniae* [4]. Detailed antimicrobial susceptibility patterns for 3GCR and CR isolates from bloodstream infections in South-East Asia are scarce. Our study contributes data on the antimicrobial susceptibilities of bacterial bloodstream pathogens, particularly for a pre-selected subpopulation of MDR bacterial isolates that would typically translate into limited treatment options for the adult and paediatric patient populations affected.

The fact that the susceptibility studies were performed at five different study sites is another strength of this study. Multi-centre studies are typically recommended to account for technical variability across study sites. The isolates characterized in this study were not collected in a systematic study and not from multiple sites per country. Instead, the phenotypic pre-selection of blood culture isolates introduces a study bias towards drug-resistant pathogens. Although this bias was deliberately sought to effectively screen a target panel of isolates with limited treatment options, it prevents simplified extrapolation to larger BSI patient populations infected with MDR GNB in SEA. Further studies are necessary to rule out potential selection biases during isolate collection in this study and to include also other antibiotic classes in order to detect also their underlying resistance prevalence.

The reason for apramycin showing best activity against the isolates in comparison to other aminoglycosides currently in clinical use most likely relates to its unique chemical structure, which is distinct from the 4,6-disubstituted 2-deoxystreptamine motif that amikacin, gentamicin, tobramycin, and plazomicin, but also etimicin, arbekacin, and many others have in common. The mono-substituted conformation of apramycin allows binding to not only the wild-type, but also the m^7G1405 methylated 16S-rRNA target site in small ribosomal subunit [12]. Most AMEs are likewise unable to inactivate apramycin, partly due to the absence of corresponding functional groups modified by AMEs in 4,6-disubstituted 2-deoxystreptamines, and partly because the unique structure of apramycin seems to evade the substrate specificity of most AMEs [10, 12]. The only known AME of potential clinical relevance that demonstrated sufficient substrate promiscuity to not only inactivate gentamicin and tobramycin but also apramycin is AAC(3)-IV [12, 20, 21]. The authors

therefore consider it conceivable to assume the four apramycin-resistant *Enterobacterales* isolates in the present study carried an *aac(3)-IV* gene, too. However, genotypic analysis of the studied isolates was beyond the scope of the current project, and further characterization by whole-genome sequencing would be required to more reliably link the various observed phenotypic resistance patterns to underlying resistance mechanisms.

Apramycin is currently in clinical development for the treatment of Gram-negative systemic infections. Our results are in support of previous connotations that apramycin may represent a new generation of therapeutic aminoglycoside antibiotics that evades the widespread antimicrobial resistance that compromises the clinical utility of 4,6-disubstituted 2-deoxystreptamines such as gentamicin, tobramycin, netilmicin, amikacin, plazomicin, arbekacin, etimicin, and others. The apramycin MIC values reported in this study are well aligned with the apramycin PKPD targets modelled previously for once-daily intravenous infusion in humans [15, 17-19]. The present study complements these prior reports by expanding our knowledge to specifically include 470 blood culture isolates and an isolate panel of well-defined geographic origin.

Aminoglycoside and polymyxin antibiotics have been used carefully in the past due to their risk of adverse effects. However, the worldwide emergence and spread of antimicrobial resistance, and in particular the increasing incidence of MDR and specifically of carbapenem-resistant GNB has continuously highlighted the clinical need for aminoglycosides or polymyxins in combination with cell-wall active agents in the treatment of critical GNB systemic infections, underscoring the importance of highly bactericidal broad-spectrum antibiotics that provide for rapid bacterial killing of high bacterial loads. Preclinical studies suggest apramycin may provide for higher drug safety when compared to other aminoglycosides [10, 13, 14]. If this were to translate into a wider therapeutic window for aminoglycoside treatment, it may further increase the clinical utility of this drug class. However, clinical evidence in patients will have yet to be provided.

Colistin has remained an important last-resort drug in the treatment of critical MDR GNB infections in adult patients, not the least because resistance to colistin is less frequently encountered than resistance to aminoglycosides. The safety and efficacy of colistin among neonates and paediatric patients, however, remain to be investigated, particularly in LMIC, where colistin resistance may be higher than elsewhere. The aminoglycoside gentamicin has remained a hallmark therapeutic in the treatment of paediatric and neonatal sepsis. However, efforts have been under way to find alternative combination therapies for the treatment of neonatal sepsis, including gentamicin-resistant infections. Recently, the substitution of gentamicin with amikacin in combination with fosfomycin has been proposed as an effective drug candidate, and the Global Antibiotic Research and Development Partnership (GARDP) has endeavoured and supported the clinical development of amikacin-fosfomycin for the treatment of neonatal sepsis in the setting of highly prevalent antimicrobial resistance [22]. It is conceivable to assume that apramycin may prove as a promising substitute in cases where amikacin resistance is reported.

In summary, our findings presented here are in support of conducting further *in vivo* studies of apramycin in animal-infection models for blood stream infections and warrant continued consideration for clinical development of apramycin.

5. Conclusions

Apramycin was found to be the most active of all drugs tested against a panel of blood culture isolates collected in South-East Asia, which included a variety of pan-aminoglycoside-, colistin-, third-generation cephalosporin-, and carbapenem-resistant Gram-negative bacteria.

Based on its high susceptibility rates and low toxicity when compared to colistin, apramycin may represent a promising next-generation aminoglycoside for the treatment of MDR Gram-negative systemic infections in South-East Asia and elsewhere.

Declarations

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Competing Interests: SNH is a co-founder of Juvabis AG. All other authors declare no conflict of interest.

Ethical Approval: Not required

Sequence Information: Not applicable

Author contributions:

MG, KB, HRvD, AJHS, EAA, TK, HHT, SV, CLL, TR, PT, and SNH conceptualized the presented work. MG, PYH, PT, AS, MS, PH, NK, TDP, THN, KH, JH, CLL, and TR performed the experiments. MG, PYH, PT, KH, SV, and SNH analysed the data. MG, JH, HRvD, AJHS, EAA, TK, SV, CLL, TR, PT, and SNH wrote the manuscript. All authors approved of the final manuscript prior to submission.

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Table 1. Antibiotic susceptibility of clinical bacterial isolates from paediatric and adult patients in South-East Asia (*n* = 470).

	Enterobacterales (*n* = 422)							***Acinetobacter* spp.** (*n* = 30)							***P. aeruginosa*** (*n* = 18)						
	S	I	R	MIC_{50}	MIC_{90}	Low	High	S	I	R	MIC_{50}	MIC_{90}	Low	High	S	I	R	MIC_{50}	MIC_{90}	Low	High
Apramycin				4	8	0.5	256				8	16	1	16				2	16	2	16
Amikacin	384	1	37	4	16	0.5	> 256	23	0	7	8	> 256	1	> 256	1	1	16	> 256	> 256	16	> 256
Gentamicin	119	4	299	64	256	≤ 0.25	> 256	3	0	27	256	> 256	1	> 256	0	0	18	> 256	> 256	256	> 256
Tobramycin	94	63	264	16	64	1	> 256	7	1	22	16	> 256	2	> 256	0	0	18	128	256	32	256
Plazomicin	353	20	49	1	8	≤ 0.25	> 256	-	-	-	4	> 256	0.5	> 256	-	-	-	> 256	> 256	16	> 256
Colistin	-	352	70	1	8	≤ 0.25	> 16	-	29	1	1	1	0.5	16	-	18	0	1	2	≤ 0.25	2

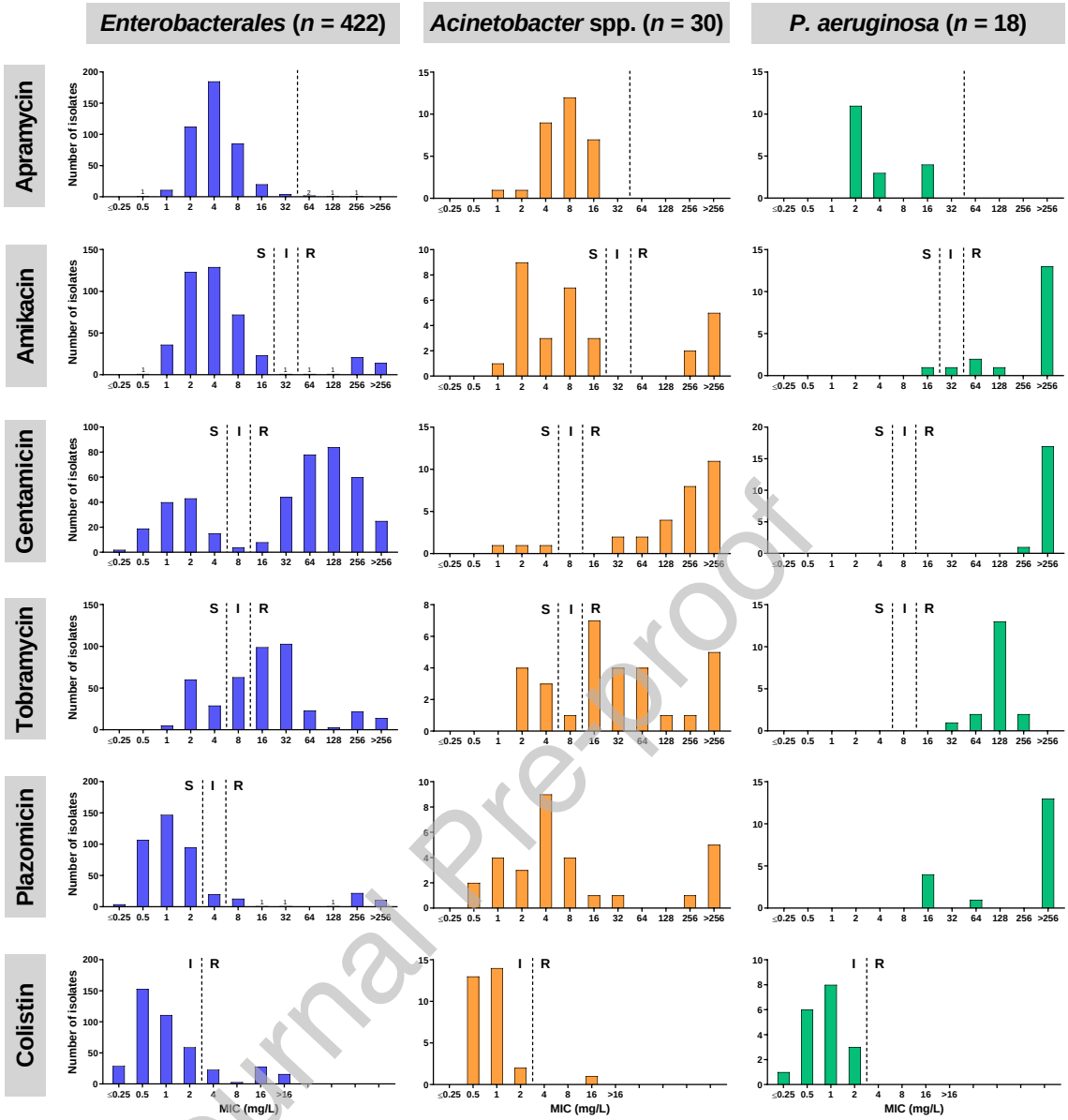

Figure 1. Minimal inhibitory concentration (MIC) distributions for *Enterobacterales, Acinetobacter* spp. and *P. aeruginosa* isolates in the South-East Asia panel tested (*n* = 470). In the apramycin graphs, a tentative resistance cut-off resembling that of amikacin is indicated by a dashed line. For amikacin, gentamicin, tobramycin, and colistin, the dashed line indicates the CLSI breakpoints. For plazomicin, the dashed line indicates the FDA-Identified Susceptibility Test Interpretive Criteria (STIC) for *Enterobacterales*. Low numbers of isolates not resulting in an easily visible bar are indicated by numbers above the MIC axis.

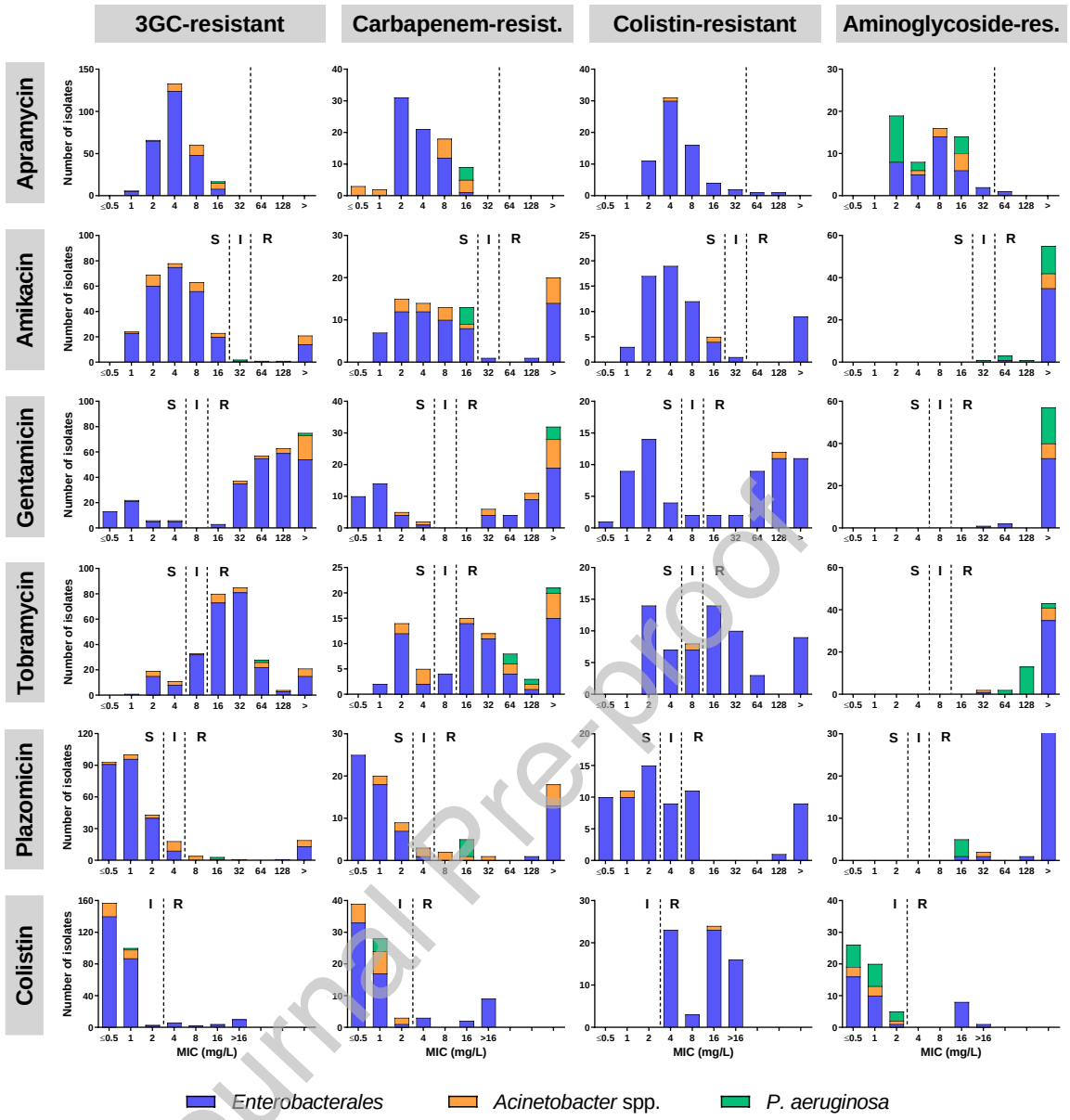

Figure 2. Minimal inhibitory concentration (MIC) distributions for phenotypic subsets of isolates. From left to right: GNB isolates resistant to at least one third-generation cephalosporin (*n* = 282), carbapenem (*n* = 84), colistin (*n* = 66), or pan-resistant to the four aminoglycosides amikacin, gentamicin, tobramycin, and plazomicin (*n* = 60). Stacked bars indicate number of *Enterobacterales* isolates in blue, number of *Acinetobacter* spp. isolates in orange, and number of *P. aeruginosa* isolates in green. In the apramycin graphs, a tentative resistance cut-off resembling that of amikacin is indicated by a dashed line. For amikacin, gentamicin, tobramycin, and colistin, the dashed line indicates the CLSI breakpoints. For plazomicin, the dashed line indicates the FDA-Identified Susceptibility Test Interpretive Criteria (STIC) for *Enterobacterales* only.