Evaluation of the RESIST ACINETO multiplex immunochromatographic assay for detection of OXA-23-like, OXA-40/58-like and NDM carbapenemase production in Acinetobacter baumannii

Mancini, Stefano ; Seth-Smith, Helena M B ; Kolesnik-Goldmann, Natalia ; Hinic, Vladimira ; Roloff, Tim ; Imkamp, Frank ; Egli, Adrian

DOI: https://doi.org/10.1093/jac/dkad253

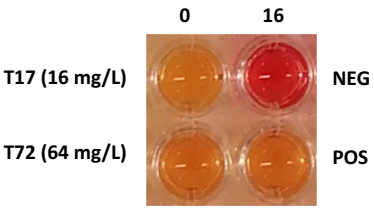

Figure 1. An example of a negative and positive test result. 0 = test solution containing no temocillin; 16 = test solution containing temocillin at a final concentration of 16 mg/L. T17, *E. coli* isolate with an MIC of 16 mg/L, T72, *K. pneumoniae* isolate with an MIC of 64 mg/L. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

resistance (MIC ≥64 mg/L) resulted in a positive test result after only 3 h; however, those with resistant MICs closer to the breakpoint (32 mg/L) required 4 h. The test was shown to be effective regardless of the Enterobacterales species, β-lactamase content and overall mechanism of resistance. This rapid test could be easily implemented in a clinical laboratory and can be set up alongside routine antimicrobial susceptibility testing methodologies (AST), but providing a result 14–20 h earlier than traditional AST and potentially sparing the use of other β-lactams such as the carbapenems. These results show that as the use of temocillin to treat Gram-negative infections becomes more commonplace, such a test can prove useful in determining targeted rather than simply empirical therapy in a relatively short time frame.

Funding

This work was financed by the Swiss National Science Foundation (grant FNS 310030_1888801).

Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

References

1 Jules K, Neu HC. Antibacterial activity and beta-lactamase stability of temocillin. *Antimicrob Agents Chemother* 1982; **22**: 453–60. https://doi.org/10.1128/AAC.22.3.453

2 Labia R, Baron P, Masson JM *et al.* Affinity of temocillin for *Escherichia coli* K-12 penicillin-binding proteins. *Antimicrob Agents Chemother* 1984; **26**: 335–8. https://doi.org/10.1128/AAC.26.3.335

3 Livermore DM, Hope R, Fagan EJ *et al.* Activity of temocillin against prevalent ESBL- and AmpC-producing Enterobacteriaceae from south-east England. *J Antimicrob Chemother* 2006; **57**: 1012–4. https://doi.org/10.1093/jac/dkl043

4 Kuch A, Zieniuk B, Żabicka D *et al.* Activity of temocillin against ESBL-, AmpC-, and/or KPC-producing Enterobacterales isolated in Poland. *Eur J Clin Microbiol Infect Dis* 2020; **39**: 1185–91. https://doi.org/10.1007/s10096-020-03844-5

5 Livermore DM, Warner M, Mushtaq S *et al.* What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. *Int J Antimicrob Agents* 2011; **37**: 415–9. https://doi.org/10.1016/j.ijantimicag.2011.01.012

6 Stewart AG, Henderson A, Bauer MJ *et al.* Activity of temocillin against third-generation cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* bloodstream isolates from a clinical trial. *JAC Antimicrob Resist* 2021; **4**: dlab192. https://doi.org/10.1093/jacamr/dlab192

7 Livermore DM, Tulkens PM. Temocillin revived. *J Antimicrob Chemother* 2009; **63**: 243–5. https://doi.org/10.1093/jac/dkn511

8 Giske CG. Contemporary resistance trends and mechanisms for the old antibiotics colistin, temocillin, fosfomycin, mecillinam and nitrofurantoin. *Clin Microbiol Infect* 2015; **21**: 899–905. https://doi.org/10.1016/j.cmi.2015.05.022

9 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Tenth Edition: M07-A11*. 2018.

10 European Committee on Antimicrobial Susceptibility Testing. Clinical breakpoints v13.0. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_13.0_Breakpoint_Tables.pdf

11 Findlay J, Gould VC, North P *et al.* Characterization of cefotaxime-resistant urinary *Escherichia coli* from primary care in South-West England 2017–18. *J Antimicrob Chemother* 2020; **75**: 65–71. https://doi.org/10.1093/jac/dkz397

12 Poirel L, Walsh TR, Cuvillier V *et al.* Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011; **70**: 119–23. https://doi.org/10.1016/j.diagmicrobio.2010.12.002

J Antimicrob Chemother 2023; **78**: 2771–2774
https://doi.org/10.1093/jac/dkad253
Advance Access publication 11 August 2023

Evaluation of the RESIST ACINETO multiplex immunochromatographic assay for detection of OXA-23-like, OXA-40/58-like and NDM carbapenemase production in *Acinetobacter baumannii*

Stefano Mancini[1]*, Helena M. B. Seth-Smith[1], Natalia Kolesnik-Goldmann[1], Vladimira Hinic[1], Tim Roloff[1], Frank Imkamp[1] and Adrian Egli[1]

[1]*Institute of Medical Microbiology, University Zurich, Gloriastrasse 28/30, 8006 Zurich, Switzerland*

*Corresponding author. E-mail: smancini@imm.uzh.ch

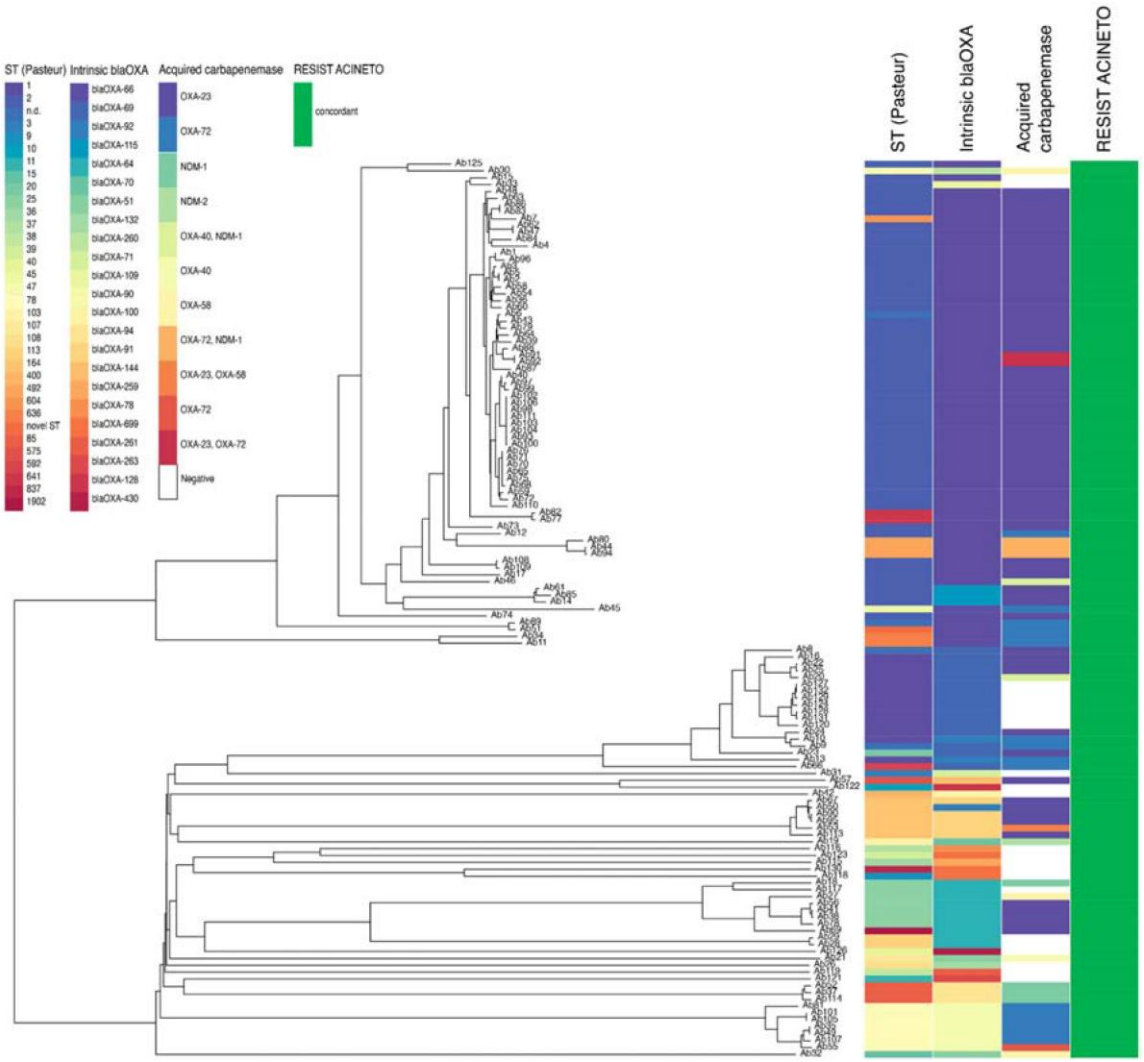

Figure 1. Phylogenetic neighbour-joining tree of *A. baumannii*. The tree was generated in Ridom SeqSphere+ based on core genes with associated metadata in columns, from left to right: ST (Pasteur), intrinsic oxacillinases identified in the genome, acquired carbapenemases and RESIST ACINETO result (green for congruent result).

Acinetobacter baumannii is a major cause of hospital-acquired infections and among the top five pathogens associated with mortality.[1] Due to its ability to rapidly acquire antimicrobial resistance traits, MDR isolates have been reported worldwide. Carbapenem-resistant *A. baumannii* (CRAB) are a particular concern, as only few treatment options, including colistin, tigecycline/eravacycline and cefiderocol, are currently available. For this reason, this pathogen has been listed by the WHO as 'priority 1' pathogen for research of new antimicrobials.[2] In this context, rapid diagnostics is crucial to guide best antibiotic treatment[3] and to prevent nosocomial transmission of CRAB. The most prevalent acquired carbapenemases in *A. baumannii* are class D oxacillinases, including the OXA-23, OXA-40 and OXA-58 groups. Other less frequently acquired carbapenemases include class A (e.g. carbapenemase variants of GES-type) and class B MBLs (e.g. NDM, VIM and IMP). Existing phenotypic methods are quite labour-intensive and exhibit variable performances in detecting carbapenemase production in *A. baumannii*.[4,5] Molecular methods including PCR or loop-mediated isothermal amplification (LAMP) assays allow for accurate detection of most prevalent carbapenemase genes but require expensive equipment.[6] Isothermal detection methods combined with lateral flow strips have been recently developed for rapid detection of the most prevalent carbapenemase genes in *A. baumannii*, but currently these assays are not commercially available.[7] Immunochromatographic lateral flow assays (LFIAs) for the detection of carbapenemase-producing *A. baumannii* isolates are available on the market, but only allow detection of single carbapenemase types, such as OXA-23 (OXA-23 *K*-SeT, Coris BioConcept, Belgium) or metallo-carbapenemases (NG-Biotech, France).[8] A recently developed type of LFIA for rapid detection of the most prevalent acquired carbapenemases in *A. baumannii*, including OXA-23, OXA-40/58 and NDM-types, is the 'RESIST ACINETO' assay (Coris BioConcept, Belgium).[9] It is important

here to note that although OXA-40 and OXA-58 belong to different families of OXA carbapenemases, their detection is combined in a single band and thus cannot be distinguished. This may represent a drawback for tracking certain types of outbreaks.

Here we evaluate retrospectively the diagnostic performance of this new assay using a collection of 131 *A. baumannii* clinical isolates (Figure 1). Fourteen strains were obtained from the Institut Pasteur's strain collection (https://www.pasteur.fr/en/public-health/biobanks-and-collections/collection-institut-pasteur-cip), while the remaining 117 clinical isolates were derived from single patients between January 2014 and December 2022 in the routine diagnostic laboratory of the Institute of Medical Microbiology at the University of Zurich. Of these, 106 exhibited carbapenem-resistant profiles, while 25 were susceptible to carbapenems. β-Lactamase-genes were detected by WGS, which was performed using our in-house available Illumina MiSeq platform with paired-end 150-nt reads. Intrinsic oxacillinases, as well as acquired carbapenem resistance markers, including carbapenemases and ESBLs, were detected using Unicycler v0.4.8 assemblies[10] in combination with ABRicate (https://github.com/tseemann/abricate) and the NCBI database.[11] All strains were typed in Ridom SeqSphere+ by MLST according to the Pasteur (ST) scheme and in addition with core-genome MLST.[12] RESIST ACINETO was performed on isolated colonies grown overnight on blood agar plates (tryptic soy agar with 5% sheep blood, bioMérieux, France) at 37°C according to the manufacturer's instructions. All genomes were submitted to the ENA (https://www.ebi.ac.uk/ena/browser) under project number PRJEB62871.

The strain collection comprised 25 carbapenemase-negative and 106 carbapenemase-producing isolates. Seventy-two of 106 isolates harboured bla_{OXA-23} (68%), 17 bla_{OXA-72} (16%), three bla_{OXA-58} and one bla_{OXA-40}, while three isolates carried two oxacillinase genes (two bla_{OXA-23}/bla_{OXA-58} and one bla_{OXA-23}/bla_{OXA-72}). Four isolates harboured bla_{NDM-1} and one bla_{NDM-2}, while the remaining carbapenemase producers harboured a combination of genes coding for NDM-1 and an oxacillinase (two bla_{NDM-1}/bla_{OXA-23}, three bla_{NDM-1}/bla_{OXA-72}). The *A. baumannii* isolates belonged to 34 different STs, with ST2 being the most prevalent (58/131; 36.7%).

RESIST ACINETO correctly identified all six carbapenemase variants, including those from the isolates producing two carbapenemases, thus exhibiting excellent sensitivity (100%). Strong bands appeared within 5–10 min of incubation in all but one case, where a faint band corresponding to NDM emerged at 15 min incubation. Nonetheless this isolate was classified as a true positive. OXA-72 was identified as a member of the OXA-40 group of OXA β-lactamases. No false positive results, which might also arise due to cross-reactivity with one of the 23 detected intrinsic OXA-51-like oxacillinases, among which OXA-66 was the most prevalent (66/131, 50.4%), were observed (specificity 100%).

A limitation of our study is that the collection of *A. baumannii* isolates is biased and reflects the epidemiological situation of the Zurich region in Switzerland, with only six different carbapenemase variants identified so far. Also, while some types were abundantly present, such as OXA-23 (68%), other globally present types, such as OXA-40, were underrepresented (1%). Moreover, in this study the performance of the RESIST ACINETO was tested on *A. baumannii* colonies grown on blood agar plates. Considering that most laboratories identify *A. baumannii* on Columbia agar or MacConkey agar plates, further studies with a more diverse collection of carbapenemase variants and isolates grown on different media are warranted to fully evaluate the robustness of the method.

In conclusion, RESIST ACINETO provides a reliable test for the detection of the most prevalent carbapenemases in *A. baumannii*. The sensitivity and specificity from isolated colonies of overnight growth is excellent (each 100%).

Acknowledgements

We thank the technician team of the IMM for dedicated help and the University of Zurich for continuous support.

Funding

This work was supported by the University of Zurich, Zurich, Switzerland.

Transparency declarations

All authors: no conflicts of interest to declare.

References

1 Resistance C A. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 2022; **399**: 629–55. https://doi.org/10.1016/S0140-6736(21)02724-0

2 Tacconelli E, Carrara E, Savoldi A *et al*. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018; **18**: 318–27. https://doi.org/10.1016/S1473-3099(17)30753-3

3 Palombo M, Bovo F, Amadesi S *et al*. Synergistic activity of cefiderocol in combination with piperacillin-tazobactam, fosfomycin, ampicillin-sulbactam, imipenem-relebactam and ceftazidime-avibactam against carbapenem-resistant Gram-negative bacteria. *Antibiotics (Basel)* 2023; **12**: 858. https://doi.org/10.3390/antibiotics12050858

4 Mitteregger D, Wessely J, Barisic I *et al*. A variant carbapenem inactivation method (CIM) for *Acinetobacter baumannii* group with shortened time-to-result: rCIM-A. *Pathogens* 2022; **11**: 482. https://doi.org/10.3390/pathogens11040482

5 Simner PJ, Opene BNA, Chambers KK *et al*. Carbapenemase detection among carbapenem-resistant glucose-nonfermenting Gram-negative bacilli. *J Clin Microbiol* 2017; **55**: 2858–64. https://doi.org/10.1128/JCM.00775-17

6 Haldorsen BC, Janice J, Samuelsen O. Evaluation of the Amplex eazyplex(R) SuperBug Acineto test for detection of acquired OXA and NDM carbapenemases in *Acinetobacter* spp. *J Glob Antimicrob Resist* 2021; **24**: 340–1. https://doi.org/10.1016/j.jgar.2021.01.019

7 Hu S, Niu L, Zhao F *et al*. Identification of *Acinetobacter baumannii* and its carbapenem-resistant gene $bla_{OXA-23-like}$ by multiple cross displacement amplification combined with lateral flow biosensor. *Sci Rep* 2019; **9**: 17888. https://doi.org/10.1038/s41598-019-54465-8

8 Mertins S, Higgins PG, Rodriguez MG *et al*. Generation and selection of antibodies for a novel immunochromatographic lateral flow test to rapidly identify OXA-23-like-mediated carbapenem resistance in *Acinetobacter baumannii*. *J Med Microbiol* 2019; **68**: 1021–32. https://doi.org/10.1099/jmm.0.001015

9 Mertins S, Higgins PG, Thunissen C *et al*. Development of an immunochromatographic lateral flow assay to rapidly detect OXA-23-, OXA-40-, OXA-58- and NDM-mediated carbapenem resistance determinants in *Acinetobacter baumannii*. *J Med Microbiol* 2023; **72**. https://doi.org/10.1099/jmm.0.001681

10 Wick RR, Judd LM, Gorrie CL *et al*. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017; **13**: e1005595. https://doi.org/10.1371/journal.pcbi.1005595

11 Feldgarden M, Brover V, Haft DH *et al*. Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. *Antimicrob Agents Chemother* 2019; **63**: e00483-19. https://doi.org/10.1128/AAC.00483-19

12 Higgins PG, Prior K, Harmsen D *et al*. Development and evaluation of a core genome multilocus typing scheme for whole-genome sequence-based typing of *Acinetobacter baumannii*. *PLoS One* 2017; **12**: e0179228. https://doi.org/10.1371/journal.pone.0179228

J Antimicrob Chemother 2023; **78**: 2774–2776
https://doi.org/10.1093/jac/dkad261
Advance Access publication 23 August 2023

Detection of *cfr* in *Klebsiella pneumoniae* from pig feed in China

Jing Wang[1,2,3]†, Yue Jiang[1,2]†, Yu-Qi Tian[1,2], Yan-Ying Qin[1,2], Xinan Jiao[1,2,3] and Zhi-Ming Pan[1,2,3]*

[1]*Jiangsu Key Laboratory of Zoonosis/Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou 225009, China;* [2]*Key Laboratory of Prevention and Control of Biological Hazard Factors (Animal Origin) for Agrifood Safety and Quality, Ministry of Agriculture of China, Yangzhou University, Yangzhou 225009, China;* [3]*Joint International Research Laboratory of Agriculture and Agri-Product Safety, Yangzhou University, Yangzhou 225009, China*

*Corresponding author. E-mail: zmpan@yzu.edu.cn
†These authors contributed equally to this article.

The multiresistance gene *cfr* encodes a 23S rRNA methyltransferase that confers resistance to five classes of antimicrobial agents widely used to treat infections in humans and animals, including oxazolidinones (limited to linezolid), phenicols, lincosamides, pleuromutilins and streptogramin A. It has been globally disseminated among Gram-positive bacteria such as *Staphylococcus* and *Enterococcus* from animals, food products, humans and the environment, and has occasionally been identified in some Gram-positive bacteria such as *Bacillus*, *Macrococcus*, *Jeotgalicoccus* and *Streptococcus*, and Gram-negative bacteria including *Escherichia coli*, *Proteus*, *Providencia rettgeri*, *Morganella morganii*, *Pasteurella multocida*, *Leclercia adecarboxylata*, *Vibrio diabolicus* and *Salmonella* from food-producing animals, pig feed or seafood in China.[1–4] Here we report the first detection of *cfr* in *Klebsiella pneumoniae* from pig feed in China.

On 23 September 2022, 48 samples including pig faeces ($n=30$), feed ($n=14$) and water ($n=4$) were obtained from a pig farm in Shanghai, China. Samples were incubated in LB broth for 16–24 h and then inoculated onto MacConkey agar. A colony per plate was randomly selected and a total of 45 isolates were obtained. We detected the presence of *cfr* by PCR and sequencing as previously described,[5] and found that one isolate, SH22PE16 (2.22%), from a pig feed sample was positive for *cfr*. This *cfr*-carrying isolate SH22PE16 was classified as *K. pneumoniae* by 16S rRNA gene sequencing.[6] MICs for SH22PE16 to 12 antimicrobial agents were determined using the agar dilution method or broth microdilution method (limited to colistin and tigecycline). The results were interpreted according to the clinical breakpoints for Enterobacterales (version 13) or epidemiological cut-off for *K. pneumoniae* set by EUCAST (https://www.eucast.org/). The *cfr*-positive *K. pneumoniae* isolate SH22PE16 exhibited resistance to numerous antibiotics, including ampicillin, cefotaxime, gentamicin, tetracycline, tigecycline, florfenicol, ciprofloxacin, fosfomycin and sulfamethoxazole/trimethoprim, but was susceptible to meropenem, amikacin and colistin (Table S1, available as Supplementary data at *JAC* Online).

To better characterize the *cfr*-positive *K. pneumoniae* isolate SH22PE16, the whole genome was sequenced using the Illumina NovaSeq 6000 platform combined with Nanopore MinION. The raw data were assembled using Unicycler version 0.4.3.8 and were analysed by multilocus sequence typing, resistance genes, mutations and plasmid replicons using the Center for Genomic Epidemiology pipeline (http://www.genomicepidemiology.org/). SH22PE16 belonged to ST5979, and carried one circular chromosome (5 106 356 bp) and five plasmids (pYUSHP16-1 to pYUSHP16-5; 2.5 to 218.9 kb) (Table S2). The WGS data of the *K. pneumoniae* isolate SH22PE16 are available under the BioProject ID PRJNA957058. It contained numerous resistance genes in the chromosome or plasmids, such as bla_{SHV-27}, $bla_{CTX-M-3}$, *tet*(A), *floR*, *oqxAB*, *qnrB91* and *fosA* (Table S2), and had a single mutation in *gyrA* (S83I), consistent with its susceptibility profiles. Although tigecycline resistance genes *tet*(X) and *tmexCD-toprJ* were not identified, the presence of the *tet*(A) variant in plasmid pYUSHP16-2, previously described to be associated with tigecycline resistance in *K. pneumoniae*,[7,8] may account for its resistance to tigecycline (MIC = 8 mg/L).

Among them, *cfr* and additional resistance genes (bla_{LAP-2} and *qnrS1*) were co-located on the 53 498 bp plasmid pYUSHP16-3, which could not be typeable to any known plasmid incompatibility groups. It was highly similar (>99.99% nucleotide identity and 90.92% coverage) to our previously reported *cfr*-carrying 56 309 bp plasmid pYUSHP29-3 of *L. adecarboxylata* from pig feed (GenBank accession no. CP087283) obtained from the same pig farm in 2019[2] (Figure 1a). To test the transferability of *cfr*, conjugation experiments were performed using streptomycin-