Increased variability of fetal heart rate during labour : a review of preclinical and clinical studies

Tarvonen, Mikko J.

2022-11

BJOG | An International Journal of Obstetrics and Gynaecology

Increased variability of fetal heart rate during labour: a review of preclinical and clinical studies

Mikko J. Tarvonen[1] | Christopher A. Lear[2] | Sture Andersson[3] | Alistair J. Gunn[2] | Kari A. Teramo[1]

[1]Department of Obstetrics and Gynaecology, University of Helsinki, and Helsinki University Hospital, Helsinki, Finland

[2]Fetal Physiology and Neuroscience Group, Department of Physiology, University of Auckland, Auckland, New Zealand

[3]Children s Hospital, Paediatric Research Centre, University of Helsinki, and Helsinki University Hospital, Helsinki, Finland

Correspondence
MJ. Tarvonen, Department of Obstetrics and Gynaecology, Helsinki University Hospital, Helsinki, Finland.
Email: mikko.tarvonen@hus.fi

Abstract

Increased fetal heart rate variability (FHRV) in intrapartum cardiotocographic recording has been variably defined and poorly understood, limiting its clinical utility. Both preclinical (animal) and clinical (human) evidence support that increased FHRV is observed in the early stage of intrapartum fetal hypoxaemia but can also be observed in a subset of fetuses during the preterminal stage of repeated hypoxaemia. This review of available evidence provides data and expert opinion on the pathophysiology of increased FHRV, its clinical significance and a stepwise approach regarding the management of this pattern, and propose recommendations for standardisation of related terminology.

KEYWORDS
acidaemia, cardiotocography, electronic fetal monitoring, fetal heart rate, increased variability, marked variability, neonatal morbidity, pregnancy, saltatory pattern, ZigZag pattern

Tweetable abstract: Increased fetal heart rate variability is parasympathetic-mediated and is caused by acutely deteriorating placental function.

1 | INTRODUCTION

Cardiotocographic (CTG) electronic fetal heart rate (FHR) monitoring is the gold standard for assessing fetal wellbeing during labour, although it has a very poor positive predictive value for fetal hypoxic ischemic neural injury.[1 5] In recent years, efforts have been made to improve the accuracy of fetal monitoring and the evaluation of intrapartum adaptation by emphasising that an adequate interpretation of a CTG tracing relies not only on recognition of FHR patterns but also on understanding the fetal physiology behind the patterns.[6 11]

The evaluation of FHR patterns is based on baseline FHR, and the depth, duration, timing and frequency of FHR decelerations and associated changes in FHR variability (FHRV).[12 16] Moderate levels of FHRV are associated with a well-oxygenated fetus, whereas reduced or absent FHRV is a warning sign of fetal compromise.[17 20] Intriguingly, there is growing evidence suggesting that increased FHRV, characterised by high-amplitude oscillations of FHR, may be important.[21,22] Experimental studies in fetal sheep have demonstrated that fetal compromise can be associated with transiently increased FHRV.[23 25] Recently, on the basis of intrapartal visual evaluation, increased FHRV has been associated with increased risk of fetal acidaemia at birth and early neonatal complications in human labour.[26 30] The definition and classification of increased FHRV vary in the literature and in CTG monitoring

guidelines.[29,31 33] Furthermore, the pathophysiologic changes that mediate increased FHRV during labour remain poorly understood.[34 38] There is a lack of consensus on factors associated with increased FHRV during labour.[27 30,39 42] Moreover, the clinical significance of increased FHRV is uncertain.[27,43,44]

The aims of the present review are to delineate the pathophysiology of increased FHRV, clarify the related terminology, and elucidate its potential clinical utility. We further propose that broadly there exist two hypoxaemia-related patterns of increased FHRV during labour: a pattern which has variously been called the ZigZag or saltatory pattern and is more often observed earlier in labour and not associated with deep repetitive FHR decelerations; and secondly, a pattern of increased FHRV observed in association with deep FHR decelerations in late labour. Suppression of FHRV, particularly in the presence of deep decelerations, remains an ominous sign that requires clinical attention. Nonetheless, it is increasingly being understood that this is not a universal finding in fetuses at risk of intrapartum acidaemia and hypoxic ischemic injury.[45 48] We therefore believe that a simplified definition and classification of these two patterns of increased FHRV will help to increase awareness and to alert birth attendants.

2 | FETAL ADAPTATION DURING LABOUR

During childbirth, uterine contractions result in repeated, brief reductions in uteroplacental perfusion, causing intermittent relative fetal and placental relative hypoxaemia. This reduction is associated with a transient fall in blood pH, base excess (BE) and oxygen tension, and a rise in carbon dioxide and base deficit (BD), even in normal, uncomplicated labour.[49 52] The fetus compensates for moderate to severe intrapartum stress by activating the peripheral chemoreflex, leading first to FHR decelerations, presumptively to reduce myocardial oxygen demand, and second to trigger peripheral vasoconstriction preferentially to support blood flow to the heart, brain and adrenal glands.[11,53 57] A healthy term fetus with a normally developed and functioning placenta is able to adapt to the typical frequency and intensity of uterine contractions without adverse consequences.[58] However, if the interval between the contractions is too short, or placental function is compromised, prolonged impairment of oxygen delivery may lead to tissue hypoxia, metabolic acidaemia, and persistent reduction in fetal cerebral oxygenation.[59 64] If these episodes of hypoxaemia continue, fetal cardiac output is progressively compromised, leading to fetal hypotension and hypoperfusion, potentially resulting in hypoxic ischemic brain injury.[65 71] The progressive worsening intrapartum fetal hypoxaemia can be observed as changes in baseline FHR and deeper FHR decelerations,[6,72,73] but once deeper decelerations are established there is typically little further change in FHR.[74] Increased FHRV typically develops in the early stage of fetal hypoxia[23,29,36,43] but can be seen also in FHR tracings of fetuses during the preterminal stage of repeated asphyxia (Appendix S1).[21,35,70]

3 | PATHOPHYSIOLOGY OF INCREASED FHRV: INSIGHT FROM PRECLINICAL ANIMAL STUDIES

Increased FHRV patterns are seen rarely in antenatal FHR tracings, occurring almost exclusively during the active stage of labour.[75,76] This suggests that labour-induced fetal stress, i.e. intermittent gas exchange disruption and consequent fetal hypoxaemia caused by intense uterine contractions, contributes to the intrapartum increased FHRV pattern.[29,77] Many studies in chronically instrumented fetal animals have used simulated intrapartum stress to improve clinical understanding of compensation mechanisms in the human fetus during birth, as well as the accompanying changes in FHR and FHRV.[47,78 83] Animal studies can be broadly separated into those that study sustained periods of hypoxaemia and those that study intermittent periods of repeated hypoxaemia. The latter is more characteristic of the repetitive nature of hypoxaemia during intrapartum uterine contractions. Sustained periods of hypoxaemia can occur for example during sentinel events (e.g. placental abruption, cord prolapse, uterine rupture) but here we propose that sustained periods of mild hypoxaemia may have an underappreciated role in some instances of increased FHRV.

3.1 | Sustained hypoxaemia

In 1977, Dalton et al.[78] reported increased FHRV during sustained moderate hypoxaemia in fetal sheep achieved by maternal inhalation of decreased oxygen, an observation that has been replicated multiple times.[43,84,85] This is typically observed in the presence of bradycardia; for example in the study by Parer et al.[43] FHR fell from $170 - 22$ to $139 - 21\,\text{bpm}$ after 5 minutes of hypoxaemia with a fall in mean pO_2 from 20.7 to $11.3\,\text{mmHg}$.[43] Likewise, more severe hypoxaemia (mean pO_2 from 22.4 to $5.8\,\text{mmHg}$) induced by complete umbilical cord occlusion (UCO) results in increased FHRV during the early minutes of UCO in association with marked bradycardia.[47,86] Of particular interest, mild hypoxaemia in fetal sheep is associated with increased FHRV without a marked fall in FHR.[43] Similarly, mild hypoxaemia in fetal monkeys was associated with an average fall in FHR from 199 to 178 bpm.[87,88] However, it is notable that individual fetuses that show a less pronounced fall in pO_2 displayed increased FHRV without a fall in FHR. In those that had a fall in FHR, this was often preceded by increased FHRV.[88]

3.2 | Repeated brief hypoxaemia

Repeated partial or complete UCOs have been used in fetal sheep to simulate the repetitive nature of hypoxaemia induced by uterine contractions. Each UCO is associated with a FHR deceleration, with more severe UCOs associated with deeper decelerations.[74,89,90] These studies have shown that the early stages of fetal adaptation to repetitive brief hypoxaemia

are associated with increased FHRV between FHR decelerations.[23,25,91] When UCO continues, the initial increase in FHRV diminishes and FHRV returns to baseline values. The terminal phase of UCO resulting in cardiovascular compromise and hypotension is associated with variable FHRV patterns.[92] In the study by Westgate et al.[23] in fetal sheep, two-thirds developed mild suppression of FHRV, with the remaining third showing a marked increase in FHRV. The mechanism underlying the differing FHRV patterns remains unknown.

3.3 | Autonomic origin

During the prepartum period, in a healthy normoxic fetus, FHRV is complexly and constantly regulated by both the sympathetic and parasympathetic nervous systems,[78,79] which are integrated at the sinoatrial node in concurrence with its own inborn rhythm.[37,38,93] Over the past decades, the pathophysiology of increased FHRV during labour has been explained by the hypothesis that during rapid hypoxaemia the fetus has insufficient time to release catecholamines, leading to impaired central organ perfusion, and a magnified autonomic response caused by instability of sympathetic and parasympathetic nervous systems.[27,36,79,84,94,95]

In contrast, more recent studies in fetal sheep have employed multiple forms of autonomic blockade during repeated UCOs to illustrate that FHRV during labour (once repetitive decelerations are apparent) is solely mediated by the parasympathetic nervous system, as recently reviewed.[96] For example, neither complete -adrenergic blockade with propranolol[83,97,98] nor chemical sympathectomy with 6-hydroxydopamine neurotoxin[37,99] reduced FHRV during repeated UCOs. In contrast, FHRV was abolished with parasympathetic blockade with either atropine sulphate or bilateral vagotomy.[38] Likewise, during sustained periods of moderate fetal hypoxaemia induced by maternal hypoxaemia, atropine but not propranolol prevented the increase in FHRV.[43,89] The mechanisms underlying the shift from dual sympathetic and parasympathetic control of FHRV during normoxia to parasympathetic dominance during both repetitive and sustained hypoxaemia are unknown but may involve feedback inhibition from high circulating catecholamine concentrations.[96] Increased FHRV during both sustained and intermittent hypoxaemia is therefore likely mediated by increased parasympathetic activity, although the upstream mechanisms driving increased parasympathetic activity are likely distinct in each scenario.

4 | HUMAN STUDIES

4.1 | Definitions and incidences

Periods of increased or high-amplitude FHRV that are occasionally observed in routine intrapartum FHR recordings have been referred to by multiple terms over the years.

Initially, these patterns were referred to as marked irregularity by Hon and Lee,[100] rapid baseline fluctuations by Caldeyro-Barcia et al.[101] and high-amplitude oscillations by Hammacher et al.[102] The current literature includes descriptions of marked variability , the saltatory pattern and the ZigZag pattern. Table 1 gives a summary of the terminology, definitions and incidences of increased FHRV used by current human studies and clinical guidelines.

Although the saltatory pattern is well known, it is notable that in a recent study of a large obstetric cohort, only six (1.0%) of the 582 CTG recordings showed increased FHRV; the duration of a single increased FHRV episode lasted between 15 and 25 minutes, and in one (0.2%) case was >25 minutes (28 minutes).[29] Furthermore, not a single increased FHRV pattern with a duration of >30 minutes was found in the cohort of 5150 childbirths.[29,30] These findings are in agreement with suggestion that the saltatory pattern, as defined by FIGO and NICE,[32,33] is almost nonexistent.[28,29]

4.2 | Association with fetal acidaemia and compromise

A recent study including 8580 births by Polnaszek et al. showed that marked variability patterns occurred in 6.7% of the 149 cases with cord blood acidaemia (UA pH <7.10).[27] Marked variability was associated with an increased risk of elevated cord blood lactate and an increased risk of respiratory distress, although no association with composite neonatal morbidity was found.[27] In their study, episodes of marked variability were most common during the final 10 minutes prior to birth, becoming progressively less common in the 2 hours studied prior to birth. The authors concluded that marked FHRV in isolation does not predict neonatal acidaemia.[27] This is in agreement with the study by O Brien-Abel and Benedetti, who concluded that a pattern of increased FHRV can be considered benign when observed in the absence of other abnormal periodic FHR changes, and in the presence of normal FHR variability before and after the high-amplitude oscillations of FHR.[44] Another recent study of 1070 fetuses who had fetal scalp blood sampled during labour showed that increased fetal scalp blood lactate level was associated with increased short-term FHRV.[103] The association was observed in all four 30-minute epochs during the last 2 hours prior to birth.[103] These findings support the concept that the early stages of intrapartum fetal hypoxaemia is associated with increased FHRV.

Recently, Tarvonen et al.[41] investigated the episodes of increased FHRV 2 minutes in duration (the ZigZag pattern) in a retrospective study of 194 CTG tracings of fetuses with low Apgar scores and their 51 healthy controls. The ZigZag pattern was associated with both cord blood acidaemia and high concentrations of cord blood erythropoietin (EPO) at birth.[41] Fetal hypoxaemia strongly stimulates EPO synthesis,[104 107] and hence high plasma EPO concentration is a marker of the severity of fetal hypoxaemia.[108,109]

TABLE 1 Definition of increased variability patterns in the FIGO, RCOG and ACOG cardiotocography interpretation guidelines and related studies

CTG guideline and study	Increased FHRV pattern	Definition	Classification	Reference on which definition and classification are based	Incidence (%)
FIGO (2015)[32]	Saltatory pattern	A bandwidth value >25 bpm	Pathological if >30 min	Nunes et al. (2014)[95]: four cases with a prolonged saltatory pattern lasting for >20 min during the last 30 min before birth from databases of 13 859 CTG tracings	0.03
RCOG (NICE) (2017)[33]	Saltatory feature	Baseline variability amplitude range > 25 bpm	Non-reassuring if 15 25 min. Abnormal if >25 min	NICE (2017)[33]: The Guideline Committee decided that the time cut-off between the non-reassuring and abnormal categories would be 25 bpm for >25 min because it is easy for clinicians to remember. The 15 min was introduced to avoid unnecessary interventions based on the presence of a single feature	
ACOG (NICHD) (2010)[129]	Marked variability	Amplitude range >25 bpm	No required duration mentioned. Analysed in 10-min epochs. Category II pattern in 3-tiered classification of FHR abnormalities	NICHD (1997)[130]: electronic fetal heart rate monitoring: research guidelines for interpretation. National Institute of Child Health and Human Development Research Planning Workshop	
Cibils (1976)[144]	Saltatory pattern	Variability of >25 bpm	No required duration mentioned	Analysis of 1304 CTG tracings.[144] FHR oscillation frequencies by Hammacher et al.[102]	7.8
O Brien-Abel & Benedetti (1992)[44]	Saltatory pattern	Amplitude changes of >25 bpm	Oscillatory frequency of >6/min for 1 min	Analysis of 433 CTG tracings.[44] Not specified on which the definition is based	2.3
Polnaszek et al. (2020)[27]	Marked variability	Fluctuations in FHR amplitude of >25 beats bpm based on 10-min epochs	No required duration mentioned	Analysis of 8580 CTG tracings.[27] For the definition, see ACOG guideline.[129]	4.5
Gracia-Perez-Bonfils et al. (2021)[28]	ZigZag pattern	Increased bandwidth of the FHR baseline (>25 bpm)	From 1 to 30 min. Differs from the saltatory pattern also in uniformity of the trace	Analysis of 500 CTG tracings.[28]	1 min in 30.1 2 min in 8.9
Tarvonen et al. (2021)[29,30,41,42]	ZigZag pattern	FHR baseline amplitude changes of >25 bpm	2 min	Analysis of 245 CTG tracings and cord blood EPO measurements.[41] Analysis of 4988 term CTG tracings.[29] Analysis of 5150 preterm, term and post-term CTG tracings.[30,42]	13.1 11.7 11.3

Abbreviations: ACOG, The American College of Obstetricians and Gynecologists; CTG, cardiotocography; EPO, erythropoietin; FHR, fetal heart rate; FIGO, The International Federation of Gynaecology and Obstetrics; NICE, National Institute for Health and Care Excellence; NICHD, Eunice Kennedy Shriver National Institute of Child Health and Human Development; RCOG, The Royal College of Obstetricians and Gynaecologists.

Further work by Tarvonen et al.[29] has highlighted the association between the ZigZag pattern and FHR decelerations. The presence of increased FHRV or late decelerations, or both, in the CTG recordings during the last 2 hours of labour has been shown to increase by three-fold the likelihood of severe hypoxaemia-related complications (i.e. UA pH <7.10 and/or BE < 12.0 mEq/l and/or 5-minute Apgar score <4 and/or intubation for resuscitation and/or grade II/III neonatal encephalopathy) in newborn infants. A CTG recording with both ZigZag pattern and late decelerations occurred in 76.9% (123/160) of cases with severe neonatal complications but in only 5.6% (201/3620) of cases with no complications.[29] Strikingly, in the vast majority of cases, a rapid transition was observed from an initially normal or reassuring FHR trace without decelerations, to the pattern of increased FHRV and the subsequent appearance of late decelerations. The median time between the end of the first ZigZag episode and the onset of late decelerations was 9 minutes.[29] Two previous case reports, and one study with a population of high-risk patients, further support the concept that the concurrent occurrence of increased FHRV and late decelerations indicates an increased risk of severe hypoxaemia.[34,35,110]

C-terminal Strep-tag II tail was cloned between the EcoRI and BamHI sites in pEPSA5 vector (primers SA0205 pEP fw and SA0205 pEP rv, Supplementary Table S1). The resulting plasmid pKTH3813 was verified by sequencing and then transferred from E. coli DH5α first to S. aureus RN4220 and then into the Newman strain to yield strains RH7777 and RH7781, respectively.

Construction of strain for microscopy and localisation of GFP-LytU in the cell. pWKD56f E. coli-S. aureus shuttle plasmid[16] was modified for expression of GFPuv4-LytU in S. aureus and determination of LytU localisation pattern in the cell as follows. The NotI-PstI fragment of pWKD56f containing the Pxyl-gfpuv4 construct was PCR-amplified with the Pxyl-gfp4 primers (Supplementary Table S1) and inserted into pWKD56f cut with NotI and PstI. This replacement was performed to insert AvrII and PacI restriction sites after the coding sequence of GFPuv4. The obtained plasmid pJUNK1 was then cut with AvrII and PacI to insert the PCR amplified coding sequence of LytU to which the same restriction sites were added with primers SA0205pMK4 (Supplementary Table S1) in frame with gfpuv4. The obtained plasmid pJUNK2 was then cut with PacI and PstI to add a synthetic transcription terminator t1R between the restriction sites (t1R primers, Supplementary Table S1) to yield plasmid pJUNK3. This plasmid was transformed into S. aureus RN4220 with selection for chloramphenicol resistance (10 μg/ml). pJUNK3 is a derivative of pMK4 (the accession number of pMK4 in GenBank is EU549779) with the replicon of pC194 having the copy number of 15 per cell.

Expression and purification of recombinant LytU proteins. For purification of different forms of recombinant proteins, chromosomal DNA from S. aureus Newman strain was used as the original PCR template. Primers used in this study have been listed in Supplementary Table S1 and the expression vector used was pGEX-2T to which inserts were cloned between the BamH1 and EcoR1 cloning sites. Constructs were confirmed by sequencing. Point mutants were obtained using QuikChange II site-directed mutagenesis kit (Agilent Technologies, USA) with the pGEX-2T carrying the previously cloned wild-type fragment as a template and results were confirmed by sequencing. For protein expression, all plasmids were transformed into E. coli BL21 (DE3) cells. Purification was carried out utilising GST-tag and followed by subsequent tag cleavage with thrombin and removal in size exclusion chromatography. Detailed procedures for expression and purification of the proteins for structural and catalytic activity studies are provided in Supplementary Information.

Cell fractionation. For localising LytU in cellular fractions overexpression strain RH7781 with and without 1% xylose induction was used. Cells grown in BHI medium were harvested with low-speed centrifugation, resuspended in 20 mM Tris pH 7.5 buffer, and ruptured using French Press. Lysate was cleared of cell debris by low-speed centrifugation (13 000 g, 4 °C) in 2 ml Eppendorf tubes and a second centrifugation of the supernatant was carried out in Beckman ultracentrifuge, 4 °C, 20 000 rpm, sw 41 rotor for 2h to create the membrane containing pellet which was then dissolved in buffer. Samples of all fractions were analyzed in SDS-polyacryl amide mini gels and gels were stained with Coomassie blue using standard methods. When whole cells of S. aureus strains were analyzed in protein gels a brief treatment with lysostaphin (Sigma-Aldrich, USA) was used prior to sample preparation.

Protoplast assay for LytU. S. aureus RH7781 was grown in BHI medium and 0.02% xylose was added at the cell density of Klett 70 to induce LytU-Strep expression for 1 hour after which cultures were harvested. Cells from 1 ml of culture were suspended in 0.1 ml of the protoplast buffer (20 mM potassium phosphate pH 7.5, 15 mM MgCl$_2$ and 20% sucrose) containing 0.1 mg/ml lysostaphin for 40 min at 37 °C. Adequate success of protoplast conversion (clear majority) was verified with microscopic examination and cells were then harvested by centrifugation at 5 000 rpm in an Eppendorf miniSpin Plus centrifuge for 5 min. The protoplast pellet was resuspended in 0.1 ml of protoplast buffer containing 1 mg/ml trypsin and then incubated for 40 min at 37 °C. A parallel sample was incubated in a similar manner but without trypsin. After the trypsin treatment trypsin inhibitor was added to the final concentration of 1.2 mg/ml. The protoplasts and protoplast supernatant were heated in Laemmli sample buffer for 10 min at 100 °C and samples were then analysed for LytU-Strep, PrsA and TrxA levels with immunoblotting using anti-Strep-tag, anti-PrsA and anti-TrxA antibodies, respectively.

Light and electron microscopy. Light and electron microscopy were carried out as previously described[25]. For light microscopy samples were Gram-stained. Confocal microscopy for LytU spatial localisation in the membrane was carried out as follows. S. aureus RN4220 carrying pJUNK3 was cultivated in BHI medium in the presence of chloramphenicol (10 μg/ml) and 0.5% xylose was added at the cell density of OD$_{600}$ 0.6 to induce GFPuv4-LytU. Samples were prepared for microscopy after 2 h induction. Cells from 1 ml of culture were harvested by centrifugation, washed once with 1 ml of PBS and resuspended in the same volume of PBS. A small drop of the cell suspension (2 μl) was spotted on a microscope slide, covered with a cover slip, and sealed with nail polish. The microscopy of the sample and non-induced control was performed with a Leica TCS-SP5 confocal laser-scanning microscope with excitation at 488 nm.

Cell wall lytic activity assay. The activity of purified LytU fragments against whole S. aureus cells was carried out using a Bioscreen C apparatus (Growth Curves, Finland) and autolysis experiment procedure as previously described[35]. Substrate cells were in this study grown either to logarithmic growth phase (OD 0.3) or late stationary phase (fresh overnight cultures) prior to harvesting and substrate sample preparation. Samples were prepared in 20 mM Tris-HCl pH 7.0 buffer. Final concentrations of cations or EDTA were 0.05 mM and the final concentration of exogenous LytU, except for controls, was 4 μM.

Pentaglycine cleavage Pentaglycine cleavage by recombinant proteins was carried out in multiple independent series of incubation samples and normalized to the activity of LytU·He with one Zn ion. For protein comparison, incubations included 0.03–0.04 mM purified protein and 1–2 mM pentaglycine (Sigma-Aldrich) in PBS buffer. ZnCl$_2$, CuCl$_2$ and CoCl$_2$ solutions were added as respective sources of metal ions to the desired protein-ion ratio. All incubations had 90% v/v D$_2$O as a solvent for water signal suppression, and their total volume was 300 l. Before the NMR analysis, reactions were incubated, in their linear range, for up to 70 hours and quenched by heating the samples for 15 min at 85 °C.

All incubations were carried out at 37 °C except when different temperatures were tested. Sodium phosphate buffers (150 mM) were used to test the effect of different pH. Before NMR analysis 0.5 mM trimethylsilyl propanoic acid (TSP) was added as a standard. Reaction progress and product formation were observed by recording a ^{1}H spectrum at 37 °C with a Varian INOVA 800 MHz spectrometer manually or with a Bruker AVANCE 600 MHz spectrometer equipped with an autosampler. Data were processed with Bruker TopSpin 3.5 and integrated with Amix 3.9.12.

Assignment of pentaglycine and its cleavage products was performed with a ^{1}H, ^{13}C HMBC experiment in PBS buffer having 99% v/v D$_2$O as a solvent. These data were acquired at 37 °C on a Bruker AVANCE III HD 800 MHz spectrometer, equipped with a TCI ^{1}H/^{13}C/^{15}N cryoprobe. The stability of the substrate pentaglycine over the time span of the activity assay was studied with a control experiment presented in Supplementary Fig. 8.

Isothermal titration calorimetry. ITC experiments were carried out at 35 °C using a MicroCal VP-ITC microcalorimeter. Purified catalytic domains of LytU were dialyzed extensively against cacodylate (Note: Extremely hazardous substance) buffer (50 mM cacodylic acid, 100 mM NaCl, pH 7.4) to remove phosphate ions of the original purification buffer and subsequently concentrated. Titrations were carried out with 50 M protein in the calorimeter cell and syringe contained 800 M ZnCl$_2$ dissolved in the final cacodylate dialysis buffer. Typical injection volume was 7 l, a spacing between injections was 300 s, and stirring speed was 300 rpm. Baseline was obtained by titrating zinc chloride into the dialysate without protein and was subtracted from the sample titration experiments. The protein-zinc binding isotherms were generated by the nonlinear least-square fitting method of the data and employing a two-site model with MicroCal Origin 7 software. Thermodynamic parameters were obtained as mean values of at least two independent measurements.

Protein structure determination. NMR samples had a protein concentration of 0.5 mM in 20 mM Bis-Tris pH 6.5 buffer, and contained 7% D$_2$O, except for histidine-optimized ^{1}H, ^{15}N HMBC spectra, where water was fully substituted with D$_2$O. The tautomeric state of histidines and the zinc coordination mode were derived from the cross peak patterns[37] observed in histidine-optimized ^{1}H, ^{15}N HMBC spectra acquired from the 0, 1 Zn and 2 Zn forms of LytU. For zinc-coordinating histidines, this analysis was in accord with the coordination mode determined from the C$^\gamma$,C$^{\delta 1}$ chemical shift difference[38].

LytU(49–192) chemical shift assignment has been described previously[39]. NOESY spectra for structure determination were acquired at 35 °C on a Varian INOVA 800 MHz spectrometer equipped with a cryogenic probe head. Distance restraints were derived from 3D ^{1}H, ^{15}N NOESY-HSQC, ^{1}H, ^{13}C NOESY-HSQC and histidine nitrogen frequency-optimized 2D ^{1}H, ^{15}N NOESY-HSQC spectra. Structures were determined by the automated procedure in Cyana 2.1[40] with the KEEP option for four manually assigned NOE peaks in both the 1 Zn and 2 Zn calculations. These assignments restrain distances between the first and the second β strands. We included one zinc in both structure calculations, with four restraints: the distance of H76 N$^\delta$, D80 O$^{\delta 1}$ and H159 N$^{\delta 1}$ to Zn was restrained to 2.0–2.2 and H76/H159 C$^\gamma$ to Zn to 4.1–4.3 and 3.0–3.2, respectively. Water is likely to complete the coordination sphere, but we found no evidence for a stable water molecule in the NOE spectra. Hence, no restraints for a fourth ligand were used. Out of 300 structures generated with Cyana, ten with the lowest target function were selected for subsequent AMBER 14[41] minimization in explicit solvent. The atomic coordinates and structural restraints for 1 Zn and 2 Zn protein structures have been deposited in the Protein Data Bank (PDB) with the accession codes 5KQB and 5KQC.

Data availability. The accession codes for atomic coordinates and structural restraints of 1 Zn and 2 Zn protein structures in the Protein Data Bank (PDB) are 5KQB and 5KQC, respectively.

References

1. Boucher, H. W. Challenges in anti-infective development in the era of bad bugs, no drugs: a regulatory perspective using the example of bloodstream infection as an indication. Clin. Infect. Dis. 50(Suppl 1), S4–9, doi:10.1086/647937 (2010).
2. Hanai, H. et al. Activated cell wall synthesis is associated with vancomycin resistance in methicillin-resistant Staphylococcus aureus clinical strains Mu3 and Mu50. J. Antimicrob. Chemother. 42, 199–209 (1998).
3. Livermore, D. M. Antibiotic resistance in staphylococci. Int. J. Antimicrob. Agents 16(Suppl 1), S3–10 (2000).
4. Pietiainen, M. et al. Transcriptome analysis of the responses of Staphylococcus aureus to antimicrobial peptides and characterization of the roles of vraDE and vraS in antimicrobial resistance. BMC Genomics 10, 429, doi:10.1186/1471-2164-10-429 (2009).
5. Kuroda, M. et al. Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in Staphylococcus aureus. Mol. Microbiol. 49, 807–821 (2003).
6. Patron, R. L., Climo, M. W., Goldstein, B. P. & Archer, G. L. Lysostaphin treatment of experimental aortic valve endocarditis caused by a Staphylococcus aureus isolate with reduced susceptibility to vancomycin. Antimicrob. Agents Chemother. 43, 1754–1755 (1999).
7. Kokai-Kun, J. F., Walsh, S. M., Chanturiya, T. & Mond, J. J. Lysostaphin cream eradicates Staphylococcus aureus nasal colonization in a cotton rat model. Antimicrob. Agents Chemother. 47, 1589–1597 (2003).
8. Kokai-Kun, J. F., Chanturiya, T. & Mond, J. J. Lysostaphin as a treatment for systemic Staphylococcus aureus infection in a mouse model. J. Antimicrob. Chemother. 60, 1051–1059, doi:10.1093/jac/dm347 (2007).
9. Kokai-Kun, J. F., Chanturiya, T. & Mond, J. J. Lysostaphin eradicates established Staphylococcus aureus biofilms in jugular vein catheterized mice. J. Antimicrob. Chemother. 64, 94–100, doi:10.1093/jac/dp145 (2009).
10. Placencia, F. X., Kong, L. & Weisman, L. E. Treatment of methicillin-resistant Staphylococcus aureus in neonatal mice: lysostaphin versus vancomycin. Pediatr. Res. 65, 420–424, doi:10.1203/PDR.0b013e3181994a53 (2009).