Exometabolome Analysis Identifies Pyruvate Dehydrogenase as a Target for the Antibiotic Triphenylbismuthdichloride in Multiresistant Bacterial Pathogens

Background: New antimicrobial targets and compounds against resistant pathogens are urgently needed. Exometabolomic and enzymologic studies identified the antimicrobial compound triphenylbismuthdichloride as an efficient inhibitor of the bacterial pyruvate dehydrogenase complex (PDHC).

Conclusion: The bacterial PDHC has attractive properties as an antimicrobial target.

Significance: We suggest that metabolomics can be very useful for studying the modes of action of antimicrobial compounds.

The desperate need for new therapeutics against notoriously antibiotic-resistant bacteria has led to a quest for novel antibacterial target structures and compounds. Moreover, defining targets and modes of action of new antimicrobial compounds remains a major challenge with standard technologies. Here we characterize the antibacterial properties of triphenylbismuthdichloride (TPBC), which has recently been successfully used against device-associated infections. We demonstrate that TPBC has potent antimicrobial activity against many bacterial pathogens. Using an exometabolome profiling approach, a unique TPBC-mediated change in the metabolites of Staphylococcus aureus was identified, indicating that TPBC blocks bacterial pyruvate catabolism. Enzymatic studies showed that TPBC is a highly efficient, uncompetitive inhibitor of the bacterial pyruvate dehydrogenase complex. Our study demonstrates that metabolomics approaches can offer new avenues for studying the modes of action of antimicrobial compounds, and it indicates that inhibition of the bacterial pyruvate dehydrogenase complex may represent a promising strategy for combating multidrug-resistant bacteria.

Antibiotics have been extremely useful for combating bacterial infections, but the currently available compounds are becoming increasingly ineffective because of the rising numbers of bacterial strains resistant to most or even all clinically available antibiotics. Methicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci (VRE),3 enterobacteria with extended-spectrum β-lactamases, multidrug-resistant Mycobacterium tuberculosis, and fluoroquinolone-resistant Clostridium difficile have dramatically increased over the last years with regular outbreaks that are hardly controllable even with strict hygiene regimes (1–3).

In recent years, it has become difficult to identify new inhibitors for the currently available targets, such as peptidoglycan biosynthetic enzymes, ribosome, gyrase, or folic acid biosynthesis. In addition, many genomics-based approaches for identifying novel targets for antibiotics have been disappointing. More sophisticated screening strategies have yielded some promising results recently (4–8), and new therapeutic concepts, such as the development of anti-virulence drugs (9, 10), the induction of bacterial programmed cell death (11), or the use of host-encoded defense peptides (12, 13), are increasingly the focus of research efforts. Nevertheless, of the small numbers of new antibiotics awaiting approval for clinical use in the near future, most are variants of ancient antibiotic classes (14, 15).

Although finding new antimicrobial compounds is a very challenging task, identifying the mode of antimicrobial action has been equally difficult. This holds particularly true for antibiotics with strong bactericidal activities that lead to an almost simultaneous halt of most vital processes in a bacterial cell, thereby limiting possibilities for elucidating what triggered the event. Genome-wide transcriptional or translational profiles are increasingly used to define signatures of gene or protein expression that are indicative of a particular type of target (16–20).
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18). However, such profiles often represent an indirect stress response rather than a direct consequence of the inhibitory event, which limits their use for identifying the mode of action of unknown compounds against new target structures (19). Metabolomics is a novel technology permitting a simultaneous qualitative and quantitative analysis of small metabolic intermediates and products by NMR- or MS-based techniques, which allows for a direct view of changes in vital metabolic pathways. Studying the total sets of extracellular metabolites has been defined as exometabolome profiling (quantitative) or footprinting (qualitative) (20). Although metabolomic analyses have so far mostly been used to study human diseases (21, 22) or to monitor metabolic changes in bacteria during different environmental conditions and in mutants (23–26), such strategies have only rarely been used to elucidate the modes of action of new antimicrobial compounds (27).

In this study, we analyzed antibacterial activities of the synthetic compound triphenylbismuthdichloride (TPBC), which has proven efficacy in preventing catheter-associated infections (28, 29). TPBC was found to have strong antimicrobial activity against major bacterial pathogens, among them many antibiotic-resistant strains, such as methicillin-resistant *S. aureus* and VRE. Using combined exometabolomic and enzymologic approaches, TPBC was shown to block the bacterial pyruvate dehydrogenase complex (PDHC), thereby abrogating central metabolic activities.

**EXPERIMENTAL PROCEDURES**

Bacterial Strains and Growth Conditions—Strains used in this work include *S. aureus* Sa113, *S. aureus* COL (methicillin-resistant *S. aureus*), *S. aureus* Newman, *Staphylococcus epidermidis* O-47, *Bacillus subtilis* DB2, *Enterococcus faecalis* VRE366, *Enterococcus faecium* VRE392, *Pseudomonas aeruginosa* MPA01, and *Escherichia coli* K12. Cultures prepared for metabolomic analyses were grown in modified RPMI 1640 medium (Sigma R7509) containing 2 mM L-glutamine and trace elements (69 μg/liter ZnCl₂, 99 μg/liter MnCl₂.4H₂O, 6 μg/liter H₃BO₃, 350 μg/liter CoCl₂, 2 μg/liter CuCl₂, 24 μg/liter NiCl₂.7H₂O, and 36 μg/liter Na₂MoO₄.2H₂O). For all other experiments, strains were grown in BM medium, containing 1% casein peptone, 0.5% yeast extract, 0.5% NaCl, 0.01% K₂HPO₄.3H₂O, and 0.1% glucose.

Determination of Antimicrobial Activities and Toxicity—Antibiotic stock solutions were prepared with appropriate solvents (e.g., TPBC was dissolved in DMSO based on its relatively high octanol/water coefficient log P value of 6.94 (see the TDR Targets Database Web site). Bacterial control cultures were incubated with equivalent amounts of corresponding solvent. Minimal inhibitory concentrations (MICs) for bacterial strains were determined using a standard protocol (30). Briefly, ~10⁶ bacteria were added to 5 ml of BM containing serially diluted inhibitory molecules. Cultures were incubated at 37 °C and shaken for 24 h, and *A*₅₇₈ was measured to determine antibiotic concentrations leading to 50% reduced growth. Human monocytic MM6 cells were precultured in VLE RPMI 1640 medium (Biochrom AG) containing 1× nonessential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin, 1 mm oxaloacetate, 0.45 mm pyruvate, and 0.2 units/ml insulin. After 48 h of incubation, cells were harvested by centrifugation (250 × g for 5 min at room temperature) and resuspended in an appropriate volume of fresh medium. ~1–2 × 10⁶ cells/ml were incubated for 3 h with increasing concentrations of inhibitor, and the percentage of dead cells was calculated after trypan blue staining and subsequent counting of stained and non-stained cells. IC₅₀ values were defined as antibiotic concentrations that lead to 50% reduction in viability.

Quantification of Extracellular Metabolites by ¹H NMR—Culture supernatants of untreated and treated *S. aureus* cultures were prepared as described above. Antibiotics were added when *A*₅₇₈ = 0.5 was reached, and this time point was defined as the starting point (0 h). Supernatants were filter-sterilized (0.22-μm pore size). The samples were then prepared for ¹H NMR analysis as described previously (31). Briefly, 200 μl of phosphate buffer (pH 7.0, 0.2 M) were mixed with 400 μl of sample in a 5-mm NMR tube, and spectra were recorded using a Bruker®Avance II 600-MHz spectrometer operating with TOPSPIN 2.0 (Bruker®Biospin). Metabolites were identified using pure reference compounds and quantified using signal integration in relation to the internal standard trimethylsilylpropionic acid-d₄ (1 mM). The identity of the key metabolite pyruvate was confirmed by GC/MS analysis as described previously (32).

Preparation of Cell-free Extracts—Cell lysates from *E. coli* were prepared by resuspending fresh cells in 2.5 ml of MOPS buffer (150 mM, pH 7.4) containing 10 μg/ml DNase I (Fermentas)/g cell wet weight. The resuspended cells were subjected to three subsequent rounds of disruption using a French press (Aminco) at 20,000 p.s.i. Insoluble components were removed from the extracts by centrifugation (15,000 × g for 20 min at 4 °C), and the clear culture supernatants were filter-sterilized and used immediately for enzymatic assays. *S. aureus* Newman cells were resuspended in 2.5 ml/g cell wet weight MOPS buffer (50 mM, pH 7.4), and 2,250 μl of the suspension were mixed with 750 μl of lysostaphin (0.025 mg/ml; Genmedics) and 30 μl of DNase I (10 mg/ml; Fermentas). Enzyme-containing suspensions were incubated for 60 min at 37 °C, and 3 × 1 ml of lysate was transferred into separate screw top polystyrene tubes each containing 400 mg of glass beads (G1145; Sigma). Cells were mechanically disrupted using a FastPrep-24 instrument (MP Biomedicals Europe) in four subsequent cycles of 20 s at lowest speed (4.0). Each cycle was interrupted for at least 30 s to avoid overheating of the sample. Insoluble components were finally removed from the extracts by centrifugation (14,000 × g for 10 min at 4 °C). 4 × 500 μl of the resulting filter-sterilized lysate were subjected to ultracentrifugation for 1 h using a Beckman TLA-55 rotor at 50,000 rpm and 4 °C. The obtained pellets were stored on ice for 1.5 h and were carefully resuspended in a total volume of 800 μl of MOPS buffer for analysis of PDHC activity.

Purification of *E. coli* PDHC—PDHC was purified from *E. coli* cells as described previously (33). Briefly, cell lysates were subjected to ultracentrifugation (4 h at 150,000 × g). Pellets were then washed with a small amount of potassium phosphate buffer (50 mM, pH 7.5) and centrifuged at 12,000 × g for 10 min. Subsequently, pellets were redissolved again, and the process of concentration and washing was repeated twice. The PDHC-containing fraction obtained after the third round of ultracen-
trifugation was loaded onto a calcium phosphate column, and PDHC was eluted using a linear gradient of potassium phosphate buffer (0.05-0.5 M). Active fractions were pooled and concentrated by ultracentrifugation as above.

**Determination of Enzymatic Activities**—PDHC activity was assayed essentially according to the method described previously (34) and modified (35). Reaction mixtures contained 50 mM MOPS (pH 7.4), 0.2 mM MgCl2, 0.01 mM CaCl2, 0.3 mM thiamine diphosphate, 0.12 mM CoA, 2.0 mM NAD, 5.1 mM pyruvate, 0.1 mM 1-methoxy-5-methylphenazinium methyl sulfate, and 0.4 mM iodonitrotetrazolium formazan in an assay volume of 1.5 ml. Enzymatic activity was measured spectrophotometrically at 500 nm and 20 °C. Units of activity were calculated using an absorption coefficient of 12.5 mm−1 cm−1 for iodonitrotetrazolium formazan.

E1 subunit activity was determined by measuring the reduction of potassium ferricyanide (K3(Fe(CN)6)), similar to the procedure described previously (36). A single reaction contained 100 mM potassium ferricyanide (37). The amount of purified enzyme. E3 subunit activity was determined by monitoring the oxidation of dihydrolipoic acid (37). The toxicity of TPBC toward human polymyxin B, TPBC exhibited potent antimicrobial activity against Gram-negative bacteria once the outer membrane is disrupted.

**RESULTS**

**TPBC Has Potent Antimicrobial Activity against Multidrug-resistant Bacterial Pathogens**—The synthetic compound TPBC (1) (Fig. 1) was shown in the 1960s (38) to have antimicrobial activity and has hardly been further investigated since then. Only recently has TPBC been used as an additive of catheter coatings to prevent device-associated infections, which yielded clinical relevance bacterial pathogens. TPBC had antibacterial activity against clinically relevant bacterial pathogens. TPBC had antibacterial activity against Gram-positive pathogens, such as staphylococci and enterococci, including methicillin-resistant *S. aureus* and VRE strains (Table 1). The MICs of TPBC were found to be

![FIGURE 1. Chemical structures of TPBC (1), triphenylbismuth (2), bismuth-salicylate (3), and fluoropyruvate (4).](image)

**TABLE 1**

| Organism                  | Inhibitory concentrations<sup>a</sup> µg/ml |
|---------------------------|--------------------------------------------|
| *S. aureus* SA113         | 0.15 ± 0.01                               |
| *S. aureus* Col (MRSA)    | 0.28 ± 0.00                               |
| *S. epidermidis* O-47     | 1.92 ± 0.07                               |
| *B. subtilis* DB2         | 0.11 ± 0.05                               |
| *E. faecalis* VRE 366     | 0.16 ± 0.01                               |
| *E. faecium* VRE 392      | 1.34 ± 0.08                               |
| *P. aeruginosa* MPA01     | 23.4 ± 1.4                                |
| *E. coli* K12             | 8.84 ± 0.53                               |
| **Toxicity (IC<sub>50</sub>)** MonoMac 6 (MM6) | >100                                       |

<sup>a</sup> Means ± S.E. of three independent experiments are shown.

**TABLE 2**

Antimicrobial activities of TPBC and chemically or functionally related compounds against *S. aureus* SA113

| Compound                  | MIC<sup>a</sup> µg/ml |
|---------------------------|------------------------|
| TPBC                      | 0.15 ± 0.01            |
| Triphenylbismuth          | 0.88 ± 0.25            |
| Bismuth(III) salicylate   | >362                   |
| Fluoropyruvate            | 16.4 ± 0.2             |

<sup>a</sup> Means ± S.E. of three independent experiments are shown.

0.15 and 0.28 µg/ml for *S. aureus* and *E. faecalis*, respectively, whereas *S. epidermidis* and *E. faecium* were slightly less susceptible. The Gram-negative bacteria *P. aeruginosa* and *Escherichia coli* were also inhibited albeit at 84- and 32-fold higher concentrations compared with *S. aureus* COL (Table 1). Gram-negative bacteria are known to be less susceptible to certain antibiotics than Gram-positive bacteria because of the outer membrane, which represents an efficient diffusion barrier. When *E. coli* was incubated with subinhibitory concentrations of TPBC plus the outer membrane-permeabilizing substance polymyxin B, TPBC exhibited potent antimicrobial activity against *E. coli* (Fig. 2), indicating that TPBC can inhibit Gram-negative bacteria once the outer membrane is disrupted.

The antimicrobial activities of TPBC and of the related compounds triphenylbismuth (2) and the anti-*Helicobacter pylori* drug bismuth-salicylate (3) (Fig. 1) were compared. TPBC exhibited by far the most potent antimicrobial activity against *S. aureus*, with bismuth-salicylate having no anti-staphylococcal activity at all (Table 2). The toxicity of TPBC toward human
MM6 macrophages was above 100 µg/ml (Table 1), suggesting that TPBC may not be harmful to human cells.

Exometabolome Analysis Indicates That TPBC Blocks Bacterial Pyruvate Catabolism—The hydrophobic properties of TPBC suggested that the substance might reach the bacterial cytoplasm and may interfere with essential cellular processes. To investigate the impact of TPBC on the primary metabolism of *S. aureus*, we chose a 1H NMR-based exometabolome profiling approach, which permits the simultaneous quantification of key metabolites in culture supernatants (31). Overall changes in the exometabolome of *S. aureus* Sa113 cultures grown in synthetic minimal medium in the presence or absence of TPBC are given in Fig. 3 and supplemental Fig. S1. In the absence of inhibitory substances, culture supernatants rapidly lost glucose and amino acids and accumulated acetate, the primary catabolic product of *S. aureus* (Figs. 3 and 4, b and c). During exponential, aerobic growth, *S. aureus* is known to oxidize glucose largely to acetate and to generate additional ATP from acetyl coenzyme A (Fig. 5). Only at late growth stages, when reduced carbon sources are limited, is acetate further oxidized to CO2 (39, 40). The central glycolysis intermediate pyruvate was detectable at low concentrations with a small, transient peak at 2 h of growth (Fig. 4d). Fermentation products, such as lactate, acetone, or butandiole, were produced only at very low levels as expected for *S. aureus* growth under aerobic conditions (Fig. 4, e–g).

After the addition of TPBC, growth, glucose and amino acid consumption, and acetate accumulation were suppressed (Figs. 3 and 4, a–c). Unexpectedly, pyruvate concentrations strongly increased in cultures containing inhibitory concentrations of TPBC over the entire duration of the experiment (Fig. 4d), indicating that glucose oxidation could only proceed to the level of pyruvate, whereas later steps were blocked. In parallel, the pyruvate-derived fermentation products acetolactate, acetone,
butanediole, lactate, formate, and ethanol accumulated over time (Figs. 3 and 4, e–g), suggesting that pyruvate was in part directed into alternative pathways. Together, these data suggested that TPBC interferes with the bacterial pyruvate catabolism.

Inhibition of Bacterial Pyruvate Catabolism Is Specific Consequence of TPBC Treatment—In order to investigate whether the accumulation of pyruvate is a specific response of *S. aureus* to TPBC or a general consequence of antibiotic treatment, we extended the exometabolomic analyses with a set of antibiotics of known modes of action. Again, the antibiotics were added to the bacterial cultures during aerobic, exponential growth at inhibitory but sublethal concentrations (Fig. 6a). The ribosomal inhibitor kanamycin, the DNA gyrase inhibitor ciprofloxacin, and the tetrahydrofolic acid biosynthesis inhibitor trimethoprim all caused a reduction of glucose consumption as expected (Fig. 5B). However, these antibiotics did not induce the accumulation of pyruvate or the production of pyruvate-derived fermentation products (Fig. 6, d–g), indicating that the TPBC-mediated change in the exometabolite profile is characteristic for this compound and does not represent a general response of staphylococci to antibiotic stress.

To further verify that TPBC blocks the oxidation of pyruvate, we compared the activity of TPBC with that of fluoropyruvate (4) (Fig. 1), a known competitive inhibitor of PDHC (41). We first noted that fluoropyruvate also has inhibitory properties against *S. aureus* (Table 2), albeit at much higher concentrations than TPBC. When the impact of fluoropyruvate on metabolite composition in staphylococcal cultures was analyzed, we observed accumulation of pyruvate in a very similar fashion as found for TPBC (Fig. 6d). However, consistent with its likely role as a competitive inhibitor of other pyruvate-utilizing enzymes, such as lactate dehydrogenase (42), fluoropyruvate did not stimulate the release of the pyruvate-derived fermentation products lactate, acetoine, or butandiole. These results provided further evidence for an interference of TPBC with the bacterial pyruvate metabolism and indicated that TPBC uses an inhibitory mechanism similar but not identical to that of fluoropyruvate.

**TPBC Is Potent Inhibitor of the Bacterial PDHC**—Most strictly or facultatively aerobic bacteria convert the catabolic intermediate pyruvate into CO₂ and acetyl-CoA via the PDHC, and PDHC-generated acetyl-CoA is necessary for many biosynthetic pathways. Accordingly, the enzyme has been shown to be essential for viability of *S. aureus* (43–45). Conversely, direct inhibition of PDHC by TPBC would explain the observed accumulation of pyruvate in the exometabolome analyses and the antimicrobial properties of this compound. To explore this possibility, we prepared cell lysates from *S. aureus*, enriched PDHC by ultracentrifugation, and monitored PDHC activity according to an established protocol that relies on the pyruvate-, CoA-, and NAD⁺-/H⁺ dependent reduction of the chromogenic electron acceptor iodonitrotetrazolium (35) in the absence or presence of TPBC. Notably, TPBC blocked PDHC activity very efficiently in a dose-dependent manner (Fig. 7a). The same inhibition was observed with *E. coli* lysates or with *E. coli* PDHC, which had been isolated by chromatographic purification (Fig. 7, b–f). PDHC was also efficiently inhibited by fluoropyruvate, as expected (Fig. 6e), whereas triphenylbismuth and bismuth-salicylate did not affect PDHC activity at all. Taken together, these data confirm that TPBC inhibits very efficiently the PDHC of different bacterial kingdoms, ranging from the firmicute *S. aureus* to the γ-proteobacterium *E. coli*. Of note, inhibition of PDHC activity and of *S. aureus* growth followed similar dose-response curves (Fig. 7a).

**TPBC Is Uncompetitive Inhibitor of PDHC**—PDHC is a multicomponent enzyme complex consisting of three functional subunits that mediate (i) decarboxylation of pyruvate and for-
formation of hydroxyethyl thiamine pyrophosphate (E1 subunit), (ii) oxidation of the hydroxyethyl group by the lipoic acid cofactor and transfer of resulting acetyl group to free CoA (E2 subunit), and (iii) oxidation of dihydrolipoic acid by NADH via the flavine adenine dinucleotide cofactor (E3 subunit). Although fluoropyruvate has been described as being a competitive inhibitor of the E1 subunit of E. coli PDHC (41), we aimed at elucidating how TPBC may inhibit the PDHC. We first performed Michaelis-Menten kinetics using enriched PDHC from E. coli and inhibitory concentrations of TPBC or fluoropyruvate. Con-
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Our study demonstrates the power of metabolomics approaches for elucidating the mode of action of new antimicrobial compounds that interfere with metabolic processes and suggests that the bacterial PDHC may become useful as a potential target for antibiotics. Most of the currently employed antibiotic targets are confined to cell wall biosynthetic enzymes and precursor molecules, such as lipid II, ribosome subunits, gyrase, RNA polymerase, and folic acid biosynthetic enzymes (47). Nevertheless, recent studies have yielded previously unrecognized potential drug targets, such as the bacterial protein degradation pathway (5), the bacterial protein synthesis (4), the cell division protein FtsZ (7), the Gram-negative lipoprotein pathway (8), or the biosynthesis of siderophores (6).

The bacterial PDHC has been investigated for more than 4 decades, and a number of studies have assigned vital and essential roles to the PDHC in staphylococci (43–45) and other bacteria (48, 49). However, to the best of our knowledge, there are no reports on PDHC-specific antibiotic compounds in the available literature. The PDHC inhibitor fluoropyruvate was first described in 1954 (50) but has not been regarded as a potential antibiotic compound. This is presumably due to the relatively high concentrations required to achieve antimicrobial effects. Using a combined exometabolic and biochemical approach, we demonstrate that PDHC can be efficiently inhibited by TPBC, whose mode of action has previously remained unknown. In contrast to fluoropyruvate, TPBC inhibited PDHC in an uncompetitive fashion and at very low concentrations (>0.2 μg/ml). Because TPBC did not affect the activity of the E1 and E3 reaction, TPBC may only be able to exert its inhibitory action in the full course of the enzymatic cascade, or it may involve the E2 subunit. The inhibition of PDHC by TPBC may relate to the previously described capacity of TPBC to phenylate ketones and enols or enolate anions (51). Detailed structural analyses of PDHC, which represent a huge multicomponent protein complex (52), will be necessary to unravel the exact mode of inhibition of PDHC by TPBC. The substantially higher antimicrobial activity of TPBC compared with fluoropyruvate may be due to a more efficient inhibitory mode (uncompetitive versus competitive) and its much more pronounced hydrophobicity, which favors the penetration across the cytoplasmic membrane. PDHC is related to other α-ketoacid dehydrogenases. However, the amounts of α-keto glutarate, the substrate of α-ketoglutarate dehydrogenase, were hardly affected by TPBC (Fig. 3), indicating that this enzyme is probably not inhibited by TPBC.

TPBC has interesting antimicrobial properties against bacterial PDHCs from unrelated organisms, such as firmicutes and proteobacteria, and it has been successfully used to coat catheters as a preventive strategy against device-related infections (28, 29). Thorough toxicological studies will be necessary in the future to explore if TPBC or related compounds may become candidates for further antinfective applications. The frequent
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and successful use of bismuth(III) salicylate or related compounds against H. pylori infections demonstrates the potential of bismuth-containing organic compounds as antimicrobial drugs and indicates that toxicity is not necessarily a problem. In fact, many bismuth-containing drugs are considered safe due to their non-toxic and non-carcinogenic properties (53).

Although numerous attempts have been undertaken to elucidate the antimicrobial mechanisms of new antimicrobial substances, it remains extremely difficult to identify the major target molecule(s). We suggest that the application of metabolomic techniques may represent a powerful approach to identify novel targets of compounds with known antimicrobial properties but unknown modes of action. This approach may be particularly promising for inhibitors of enzymes that metabolize small compounds, such as pyruvate. Although we did not see characteristic metabolite changes in bacterial culture supernatants upon the addition of ribosome, DNA gyrase, or tetrahydrofolic acid biosynthetic inhibitors, detailed analyses of intracellular metabolomes may yield characteristic signatures for all classes of antibiotics in the future. Our ongoing efforts will help to further assess the power of metabolomics in antimicrobial drug discovery.

The antimicrobial properties of TPBC along with its proven efficacy in preventing catheter-associated infections put TPBC on the list of promising antibiotic lead substances. Our study represents a basis for using TPBC-derived drugs or novel compounds to target the bacterial PDHC as a new therapeutic option against highly antibiotic-resistant bacteria, such as methicillin-resistant S. aureus, VRE, and others.

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