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# Comparison of antibiotic resistance phenotypes in laboratory strains and clinical isolates of *Staphylococcus aureus*, *Salmonella* Typhimurium, and *Klebsiella pneumoniae*

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**Abstract** This study was designed to evaluate the antibiotic resistance phenotypes in wild-type *Staphylococcus aureus* (WT-SA), oxacillin-induced *S. aureus* (OI-SA), clinically-acquired antibiotic-resistant *S. aureus* (CA-SA), wild-type *Salmonella* Typhimurium (WT-ST), ciprofloxacin-induced *S.* Typhimurium (CI-ST), clinically-acquired antibiotic-resistant *S.* Typhimurium (CA-ST), wild-type *Klebsiella pneumoniae* (WT-KP), ciprofloxacin-induced *K. pneumoniae* (CI-KP), and clinically-acquired antibiotic-resistant *K. pneumoniae* (CA-KP). The resistance of WT-SA, WT-ST, and WT-KP to ampicillin, ceftazidime, and cephalotin, penicillin was increased after induction by oxacillin OI-SA, ciprofloxacin CI-ST, and ciprofloxacin CI-KP, respectively. The highest β-lactamase activities were 12 and 36 μmol/min/ml, respectively, for CA-ST and CA-KP. The EtBr residues remained high in *S.* Typhimurium (>80%) and *K. pneumoniae* (>90%) when treated with CCCP. The distinct FT–IR spectra were observed in protein region (1700–1500 $cm^{-1}$) and carbohydrate region (1200–900 $cm^{-1}$). This study would provide useful information for better understating of specific resistance mechanisms in association with β-lactamase and efflux pump activities.

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**Keywords** B-lactamase activity · Efflux pump activity · Resistance phenotype · *Staphylococcus* · *Salmonella* · *Klebsiella*

## Introduction

Over the past few decades, antibiotics have long been used to treat disease-causing bacteria. However, their chemotherapeutic misuse and overuse have led to the emergence of resistant bacteria [1]. The antibiotic-resistant bacterial infections have become of great public health concerns due to the problems with diagnosis, treatment, and prevention. The mechanisms of antibiotic resistance in bacteria include enzymatic modification (β-lactamases), decreased permeability (selective blockade), increased membrane transport (efflux pumps), altered binding site (specific receptors), and metabolic bypass (alternate pathway) [2]. The horizontal gene transfer is responsible for the spread of plasmid-encoded β-lactamases genes such as extended spectrum β-lactamase (ESBL), AmpC, and TEM-1 [3, 4]. The production of the β-lactamase contribute to antibiotic resistance by directly hydrolyzing β-lactam antibiotics. Multidrug efflux pumps are an important resistance determinants by extruding antibiotics out of bacteria [5]. The efflux pumps have a broad substrate specificity.

The development of rapid detection methods has recently received great attention with increasing the emergence of multidrug resistant bacteria. The detection of multidrug resistance is a primary step to effectively control antibiotic-resistant bacterial infections that can cause clinical treatment failure and lead to additional antibiotic resistance [6, 7]. The antibiotic susceptibility profiles are commonly used to determine the resistance phenotype of

bacteria as measured by minimum inhibitory concentrations (MICs). Molecular techniques are used for the confirmation of antibiotic resistant genes in bacteria. However, the resistance phenotypes do not always correlate with the resistance phenotypes. The discrepancies between phenotypic and genotypic antibiotic-resistant properties can cause false interpretation of antibiotic susceptibility results, ultimately leading to the inappropriate antibiotic therapy. Therefore, it is essential to assess the resistance phenotype in association with antibiotic resistance mechanisms such as β-lactamase and efflux pump activities. In this study, we aimed to compare the antibiotic resistance phenotypes in laboratory, antibiotic-induced, and clinical isolates of *Staphylococcus aureus*, *Salmonella* Typhimurium, and *Klebsiella pneumoniae*.

## Materials and methods

### Bacterial strains and culture condition

Wild-type strains of *Staphylococcus aureus* ATCC 15564 (WT-SA), *Salmonella* Typhimurium ATCC 19585 (WT-ST), and *Klebsiella pneumoniae* ATCC 23357 (WT-KP) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The stepwise selection method [8] was used to induce the antibiotic-induced *S. aureus* ATCC 15564, *S.* Typhimurium ATCC 19585, and *K. pneumoniae* ATCC 23357, which were assigned to oxacillin-induced *S. aureus* (OI-SA), ciprofloxacin-induced *S.* Typhimurium (CI-ST), and ciprofloxacin-induced *K. pneumoniae* (CI-KP), respectively. The stability of induced antibiotic resistance in OI-SA, CI-ST, and CI-KP was confirmed by prolonged cultures (>10 passages) in antibiotic-free trypticase soy broth (TSB; Difco, Becton, Dickinson and Co., Sparks, MD, USA). The clinically-acquired antibiotic-resistant strains, *S. aureus* CCARM 3080 (CA-SA), *S.* Typhimurium CCARM 8009 (CA-ST), and *K. pneumoniae* CCARM 10237 (CA-KP), were obtained from Culture Collection of Antibiotic Resistant Microbes (CCARM, Seoul, Korea). All strains were cultured aerobically in TSB at 37 °C for 20 h and then harvested by centrifugation at 3000×*g* for 20 min at 4 °C. The harvested cells were washed with phosphate-buffered saline (PBS; pH 7.2) and then used in the assays [9].

### Antibiotic susceptibility assay

The antibiotic susceptibilities of WT-SA, WT-ST, WT-KP, OI-SA, CI-ST, CI-KP, CA-SA, CA-ST, and CA-KP were determined according to microbroth dilution assay. Antibiotic stock solutions (2048 μg/ml each; ampicillin, cefotaxime, cefoxitin, ceftazidime, cephalotin, imipenem, meropenem, oxacillin, penicillin, and piperacillin) were prepared by dissolving in sterile distilled water. Each antibiotic solution (100 μl) prepared in a series of twofold dilutions in 96-well microtiter plates and then inoculated with each test strain ($10^5$ cfu/ml in 100 μl) to obtain a final concentration of 512 μg/ml. The prepared plates were incubated for 18 h at 37 °C to determine minimum inhibitory concentration (MIC), defined as the lowest antibiotic concentration with no visible growth. MIC breakpoints were used to define susceptible (S), intermediate (I), and resistant (R) strains [10].

### Lactamase inhibition assay

The susceptibilities of WT-SA, WT-ST, WT-KP, OI-SA, CI-ST, CI-KP, CA-SA, CA-ST, and CA-KP to β-lactam antibiotics were evaluated in the absence and presence of β-lactamase inhibitor (tazobactam, 4 μg/ml) [11]. MICs were determined as above mentioned.

### Nitrocefin hydrolysis assay

The β-lactamase activity of WT-SA, WT-ST, WT-KP, OI-SA, CI-ST, CI-KP, CA-SA, CA-ST, and CA-KP was measured by using a β-lactamase activity colorimetric assay kit (BioVision Inc., Milpitas, CA, USA). All strains are incubated in TSB containing 1/4 MIC oxacillin at 37 °C for 20 h. The cell-free supernatant was prepared by centrifugation at 3000×*g* for 20 min at 4 °C and incubated with 50 μl of reaction mixture containing 48 μl of b-lactamase assay buffer and 2 μl of nitrocefin at 37 °C for 30 min. The ability of nitrocefin to hydrolyze β-lactamase was measured at 490 nm using a microplate reader (Bio-Tek Instruments, Inc.). A standard curve was prepared at 0, 5, 10, 15, 20, 30, 40, and 50 nmol of hydrolyzed nitrocefin standard. The β-lactamase activity was expressed as nmol/min/ml.

### Efflux pump inhibition assay

The efflux pump activity of WT-SA, WT-ST, WT-KP, OI-SA, CI-ST, CI-KP, CA-SA, CA-ST, and CA-KP was evaluated in the absence and presence of efflux pump inhibitors (EPIs), carbonyl cyanide-*m*-chlorophenyl hydrazone (CCCP) and phenylalanine-arginine-β-naphthylamide (PAβN). All strains adjusted to 0.5 McFarland standard were incubated in PBS containing ethidium bromide (2 μg/ml, EtBr) and CCCP (1/4 MIC) for 1 h at 25 °C. The EtBr-loaded cells were suspended with 0.4% glucose in PBS and then mixed with CCCP (1/4 MIC) or PAβN (1/4 MIC). The fluorescence was measured using a RF-5301PC spectrofluoro photometer (Shimadzu, Kyoto,

Japan) at emission and excitation wavelengths of 580 nm and 500 nm, respectively.

### FT–IR analysis

FT–IR spectrometer (Excalibur series; Bio-Rad, Cambridge, MA) equipped with a UMA-500 microscope was used to evaluate the biochemical properties of WT-SA, WT-ST, WT-KP, OI-SA, CI-ST, CI-KP, CA-SA, CA-ST, and CA-KP. All cells were lyophilized prior to FT–IR analysis. FT–IR spectra were recorded in wave numbers ranging from 4000 $cm^{-1}$ to 650 $cm^{-1}$ with a resolution of 4 $cm^{-1}$.

### Statistical analysis

Data were analyzed by the Statistical Analysis System (SAS) software. All analyses were carried out in duplicate for three replicates. The general linear model (GLM) and Fisher's least significant difference (LSD) procedures were used to determine significant mean differences at $P < 0.05$.

**Table 2** Changes in antibiotic susceptibility of *Salmonella* Typhimurium in the presence of tazobactam (TB)

| Antibiotic | *Salmonella* Typhimurium[a] | | | | | |
|---|---|---|---|---|---|---|
| | WT-ST | | CI-ST | | CA-ST | |
| | w/o TB | w TB | w/o TB | w TB | w/o TB | w TB |
| Ampicillin | 8 (S)[b] | 2 | 16 (S) | 8 | >512 (R) | 32 |
| Cefotaxime | 4 (S) | 0.125 | 4 (S) | 0.5 | 4 (S) | 0.125 |
| Cefoxitin | 2 (S) | 8 | 8 (S) | 64 | 4 (S) | 16 |
| Ceftazidime | 0.25 (S) | 0.25 | 0.5 (S) | 0.25 | 1 (S) | 0.25 |
| Ceftriaxone | 0.25 (S) | 0.25 | 0.25 (S) | 0.25 | 0.125 (S) | 0.125 |
| Cephalotin | 8 (S) | 8 | 64 (R) | 32 | 32 (R) | 16 |
| Imipenem | 2 (I) | 0.5 | 2 (I) | 0.25 | 1 (S) | 0.5 |
| Meropenem | 0.25 (S) | 0.25 | 0.03 (S) | <0.03 | 0.125 (S) | 0.125 |
| Penicillin | 4 (S) | 4 | 32 (R) | 32 | >512 (R) | 8 |
| Piperacillin | 4 (S) | 2 | 8 (S) | 8 | >512 (R) | 8 |

[a] WT-ST, CI-ST, and CA-ST denote wild-type *S.* Typhimurium, ciprofloxacin-induced *S.* Typhimurium, and clinically-acquired antibiotic-resistant *S.* Typhimurium, respectively

[b] S, I, and R represent susceptible, intermediate, and resistant strains, respectively

## Results and discussion

### Changes in antibiotic susceptibilities in the presence of β-lactamase inhibitor

The MICs of β-lactam antibiotics against WT-SA, OI-SA, CA-SA, WT-ST, CI-ST, CA-ST, WT-KP, CI-KP, and CA-KP were determined in the absence and presence of β-lactamase inhibitor, tazobactam, as shown in Tables 1, 2, and 3. All strains were classified into antibiotic-sensitive (S), intermediate (I) and resistant (R) based on the MIC breakpoints [10]. After resistance induction by oxacillin or ciprofloxacin, the susceptibilities to most β-lactam antibiotics were decreased in OI-SA, CI-ST, and CI-KP (Tables 1–3). Compared to wild-type strains, CA-SA, CA-ST, and CA-KP were highly resistant to ampicillin,

**Table 1** Changes in antibiotic susceptibility of *Staphylococcus aureus* in the presence of tazobactam (TB)

| Antibiotic | *Staphylococcus aureus*[a] | | | | | |
|---|---|---|---|---|---|---|
| | WT-SA | | OI-SA | | CA-SA | |
| | w/o TB | w TB | w/o TB | w TB | w/o TB | w TB |
| Ampicillin | 8 (S)[b] | 0.25 | 64 (R) | 8 | 256 (R) | 32 |
| Cefotaxime | 2 (S) | 2 | 8 (I) | 8 | >512 (R) | >512 |
| Cefoxitin | 4 (S) | 2 | 4 (S) | 4 | >512 (R) | 256 |
| Ceftazidime | 8 (S) | 16 | 128 (R) | 128 | >512 (R) | >512 |
| Ceftriaxone | 4 (S) | 2 | 32 (R) | 32 | >512 (R) | >512 |
| Cephalotin | 0.5 (S) | 0.125 | 4 (R) | 2 | 256 (R) | 128 |
| Imipenem | <0.03 (S) | <0.03 | 0.125 (S) | <0.06 | 256 (R) | 128 |
| Meropenem | 0.25 (S) | 0.25 | 1 (I) | 1 | 128 (R) | 128 |
| Oxacillin | 0.25 (S) | 0.125 | 8 (R) | 8 | >512 (R) | 512 |
| Penicillin | 16 (R) | 0.06 | 64 (R) | 4 | 32 (R) | 32 |
| Piperacillin | 16 (S) | 1 | 16 (S) | 16 | 512 (R) | 128 |

[a] WT-SA, OI-SA, and CA-SA denote wild-type *S. aureus*, oxacillin-induced *S. aureus*, and clinically-acquired antibiotic-resistant *S. aureus*, respectively

[b] S, I, and R represent susceptible, intermediate, and resistant strains, respectively

**Table 3** Changes in antibiotic susceptibility of *Klebsiella pneumonia* in the presence of tazobactam (TB)

| Antibiotic | *Klebsiella pneumonia*[a] | | | | | |
|---|---|---|---|---|---|---|
| | WT-KP | | CI-KP | | CA-KP | |
| | w/o TB | w TB | w/o TB | w TB | w/o TB | w TB |
| Ampicillin | 256 (R)[b] | 8 | >512 (R) | 128 | >512 (R) | >512 |
| Cefotaxime | 0.125 (S) | 0.125 | 8 (I) | 0.5 | 128 (R) | 128 |
| Cefoxitin | 16 (I) | 32 | 64 (R) | 64 | 512 (R) | 512 |
| Ceftazidime | 1 (S) | <0.5 | 8 (S) | 0.5 | >512 (R) | >512 |
| Ceftriaxone | 0.25 (S) | 0.25 | 4 (S) | 0.25 | 128 (R) | 32 |
| Cephalotin | 32 (R) | 16 | 64 (R) | 64 | >512 (R) | >512 |
| Imipenem | 4 (R) | 2 | 16 (R) | 4 | 32 (R) | 2 |
| Meropenem | 0.125 (S) | 0.125 | 0.25 (S) | 0.25 | 0.5 (S) | 1 |
| Penicillin | 256 (R) | 32 | 512 (R) | 128 | >512 (R) | >512 |
| Piperacillin | 16 (S) | 4 | 16 (S) | 8 | >512 (R) | 512 |

[a] WT-KP, CI-KP, and CA-KP denote wild-type *K. pneumoniae*, ciprofloxacin-induced *K. pneumoniae*, and clinically-acquired antibiotic-resistant *K. pneumoniae*, respectively

[b] S, I, and R represent susceptible, intermediate, and resistant strains, respectively

penicillin, and piperacillin, showing MICs of more than 512 μg/ml. The results indicate that antibiotic selection pressure can induce multidrug resistant bacteria [12]. The susceptibilities of all strains tested in this study to β-lactam antibiotics were increased in the presence of tazobactam. The susceptibilities of WT-SA, OI-SA, and CA-SA to ampicillin were increased by more than eightfold (Table 1), those of WT-ST, CI-ST, and CA-SA to cefotaxime were increased by more than eightfold (Table 2), and those of WT-KP, CI-KP, and CA-KP to imipenem were increased by more than twofold (Table 3). Tazobactam could inhibit TEM, SHV and CTX-M type β-lactamases which hydrolyze the penicillins and cephalosporins [13, 14]. This indicates that β-lactamases produced by WT-SA, OI-SA, CA-SA, WT-ST, CI-ST, WT-KP, CI-KP and CA-KP might be TEM, SHV and CTX-M type β-lactamases.

The extracellular β-lactamase activities were measured in all strains treated with oxacillin. No significant change in β-lactamase activities was observed in CA-SA, WT-ST, CI-ST, WT-KP, and CI-KP (Fig. 1). This suggests that CA-SA, WT-ST, CI-ST, WT-KP and CI-KP could have

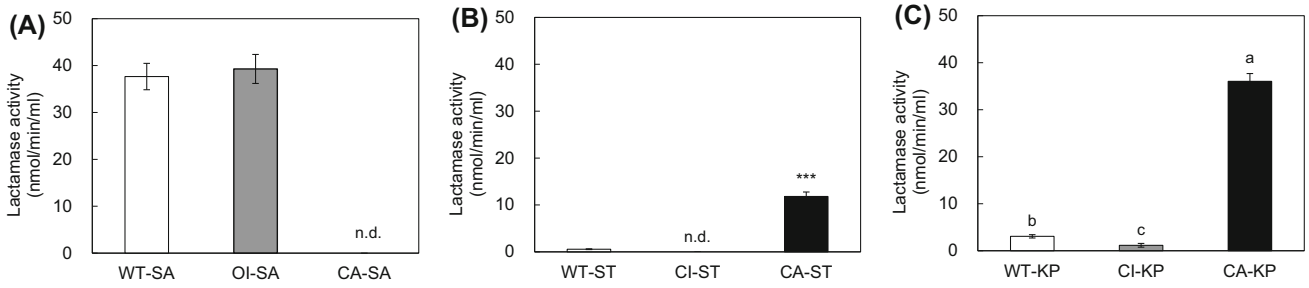


**Fig. 1** Hydrolyzing activity of β-lactamases produced by wild-type (WT), ciprofloxacin-induced (CI), oxacillin-induced (OI), and clinically-acquired antibiotic resistant (CA) *Staphylococcus aureus* (SA), *Salmonella* Typhimurium (ST), and *Klebsiella pneumonia* (KP). Means with *different letters* (**A**–**B**) on the *bars* are significantly different at $P < 0.05$. (n.d.) and (***) not detected and significantly different at $P < 0.001$

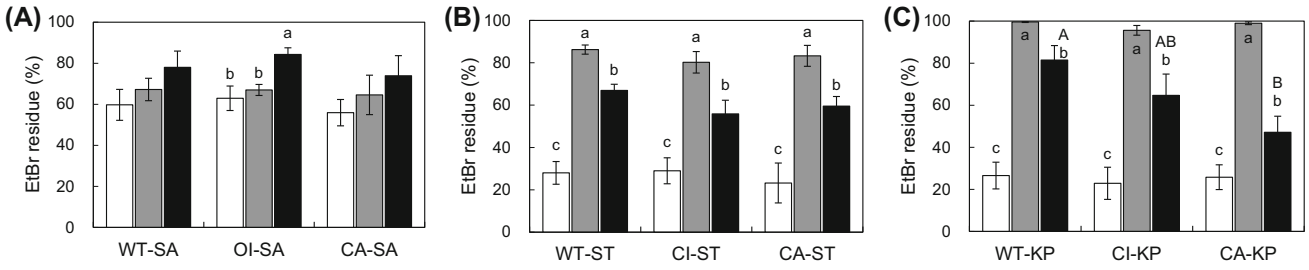


**Fig. 2** Efflux pump activity of *Staphylococcus aureus*, *Salmonella* Typhimurium, and *Klebsiella pneumonia* in the presence of none (*white square*), CCCP (*gray square*), and PAβN (*black square*). Means with *different letters* within treatment (**A**–**C**) and strains (**A**–**C**) on the bars are significantly different at $P < 0.05$. WT-SA, WT-ST, WT-KP, OI-SA, CI-ST, CI-KP, CA-SA, CA-ST, and CA-KP denote wild-type *S. aureus*, *S.* Typhimurium, *K. pneumoniae*, oxacillin-induced *S. aureus*, ciprofloxacin-induced *S.* Typhimurium, *K. pneumoniae*, clinically-acquired antibiotic-resistant *S. aureus*, *S.* Typhimurium, and *K. pneumoniae*, respectively

different resistance mechanisms such as penicillin-binding proteins (PBPs) with low affinity for β-lactamases and changes in membrane permeability [15–17]. The highest β-lactamase activities were observed in WT-SA and OI-SA, showing 38 and 39 μmol/min/ml, respectively. The β-lactamase activities of the CA-ST and CA-KP were 12 and 36 μmol/min/ml, respectively. The production of the β-lactamases was increased in WT-SA, OI-SA, CA-ST and CA-KP when exposed to oxacillin. This implies that the pre-exposure of bacteria to β-lactams can stimulate the induction of β-lactamases.

### Changes in efflux pump activities in the presence of efflux pump inhibitors

The efflux pump activities of all strains used in this study were evaluated in the absence or presence of efflux pump inhibitors (EPIs; CCCP and PaβN) (Fig. 2). No significant change in EtBr residues was observed among WT-SA, CI-SA, and CA-SA treated with CCCP (Fig. 2A). The EtBr residues of WT-SA, CI-SA, and CA-SA in the absence and presence of EPIs were more than 60%, showing low efflux pump activity. This observation implies that *S. aureus* had relatively low efflux pump activity or EPIs were less effective against Gram-positive than Gram-negative bacteria. Unlike *S. aureus* strains, the noticeable efflux activities were observed for *S.* Typhimurium (Fig. 2B) and *K. pneumoniae* (Fig. 2C). The EtBr residues were decreased to less than 30% in *S.* Typhimurium and *K. pneumoniae* treated with no EPIs, while those remained 80% and 100%, respectively, in *S.* Typhimurium and *K. pneumoniae* when treated with CCCP. Although both CCCP and PAβN could significantly decrease the efflux pump activity, CCCP is more effectively inhibit the efflux pump systems than PAβN (non-specific inhibitor) in *S.* Typhimurium and *K. pneumoniae*. The efflux-mediated antibiotic resistance is attributed to the proton motive force and substrate competition [18]. CCCP and PAβN can disrupt proton electrochemical gradient and compete with antibiotics, respectively [19]. In Fig. 3C, the EtBr residues varied in strains, showing 80, 60, and 50%, respectively, in WT-KP, CI-KP, and CA-KP. This suggests that various efflux pump systems were developed in antibiotic-resistant *K. pneumoniae* [20].

### Changes in chemical components of the bacterial cells

FT–IR was used to differentiate and characterize the chemical components of WT-SA, OI-SA, CA-SA, WT-ST, CI-ST, CA-ST, WT-KP, CI-KP and CA-KP (Fig. 3; Table 4). The spectral range was divided by wavenumber, including lipid region (3000–2800 $cm^{-1}$), protein region (OMP; 1700–1500 $cm^{-1}$), phospholipid-nucleic acid

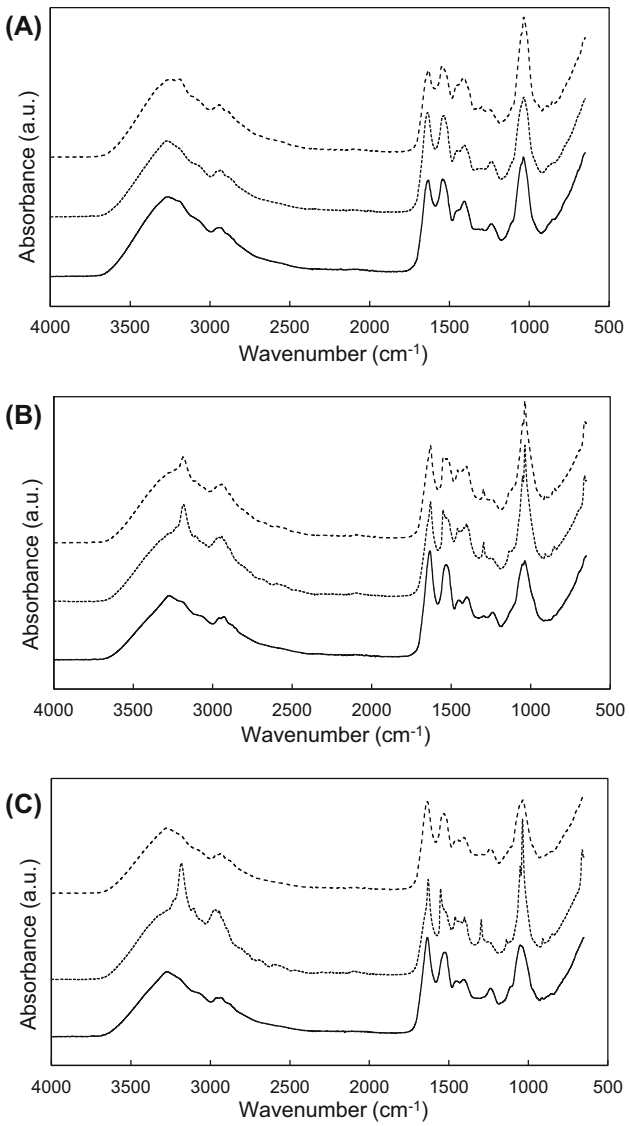


**Fig. 3** FT–IR spectra of wild-type (WT), oxacillin-induced (OI), ciprofloxacin-induced (CI), and clinically-acquired (CA) *Staphylococcus aureus* (SA), *Salmonella* Typhimurium (ST), and *Klebsiella pneumonia* (KP). a.u. arbitrary units. (*solid curve*) WT-SA, WT-ST, and WT-KP; (*doted curve*) OI-SA, CI-ST, and CI-KP; (*dashed curve*) CA-SA, CA-ST, and CA-KP

region (1500–1200 $cm^{-1}$), carbohydrate region (LPS; 1200–900 $cm^{-1}$), and fingerprint region (900–700 $cm^{-1}$) (Fig. 3) [21]. The functional groups and corresponding references were assigned to the absorbance peaks (Table 4). The highest intensities were observed at 3178, 2970, 1625, 1553, 1401, 1299, and 1125 $cm^{-1}$ corresponding to asymmetric stretching mode of $NH_2$, C–H stretch of $CH_3$ in fatty acids, amide I of α-helix structure of protein, amide II mode, symmetric COO– stretching, C–N stretching vibration, and C–H bending vibration, respectively, in all strains. Major cellular components were collected in protein region (1700–1500 $cm^{-1}$) and

**Table 4** Frequencies and assignments of absorbance peaks in FT–IR spectra of wild-type (WT), ciprofloxacin-induced (CIP), oxacillin-induced (OIAR), and clinical acquired (CAAR) *Staphylococcus aureus*, *Salmonella* Typhimurium, and *Klebsiella pneumonia*[a]

| Wave no. | *S. aureus* | | | *S.* Typhimurium | | | *K. pneumonia* | | | Band assignment |
|---|---|---|---|---|---|---|---|---|---|---|
| ($cm^{-1}$) | WT-SA | OI-SA | CA-SA | WT-ST | CI-ST | CA-ST | WT-KP | CI-KP | CA-KP | |
| 720 | 0.32 | 0.30 | 0.30 | 0.26 | 0.28 | 0.29 | 0.26 | 0.27 | 0.25 | C–H rocking of $CH_2$ |
| 960 | 0.17 | 0.18 | 0.18 | 0.16 | 0.17 | 0.18 | 0.15 | 0.13 | 0.15 | C–H rocking of $CH_2$ and $CH_3$ |
| 1036 | 0.40 | 0.40 | 0.47 | 0.34 | 0.53 | 0.49 | 0.31 | 0.56 | 0.32 | Carbohydrate C–O stretching mode |
| 1080 | 0.26 | 0.27 | 0.26 | 0.25 | 0.25 | 0.26 | 0.25 | 0.19 | 0.24 | Phosphodiester groups of DNA and RNA |
| 1154 | 0.13 | 0.13 | 0.12 | 0.14 | 0.13 | 0.14 | 0.12 | 0.10 | 0.13 | Carbohydrate C–O stretching mode |
| 1211 | 0.16 | 0.16 | 0.13 | 0.14 | 0.12 | 0.13 | 0.14 | 0.10 | 0.13 | Stretching of PO bond in phosphate |
| 1222 | 0.17 | 0.17 | 0.14 | 0.15 | 0.13 | 0.14 | 0.16 | 0.11 | 0.14 | PO bond in phosphate |
| 1303 | 0.16 | 0.16 | 0.16 | 0.15 | 0.17 | 0.16 | 0.13 | 0.16 | 0.13 | Vibration C–N of amides II |
| 1370 | 0.19 | 0.19 | 0.21 | 0.17 | 0.19 | 0.19 | 0.16 | 0.14 | 0.15 | Bending of C–H |
| 1384 | 0.22 | 0.22 | 0.23 | 0.20 | 0.23 | 0.23 | 0.18 | 0.17 | 0.18 | Symmetric stretching of COO– and $CH_2/CH_3$ |
| 1420 | 0.24 | 0.23 | 0.26 | 0.20 | 0.25 | 0.25 | 0.19 | 0.20 | 0.19 | Symmetric stretching of COO– |
| 1450 | 0.22 | 0.22 | 0.24 | 0.20 | 0.24 | 0.24 | 0.19 | 0.20 | 0.19 | C-H bending of $CH_2$; $CH_2$ scissoring |
| 1455 | 0.22 | 0.22 | 0.24 | 0.20 | 0.24 | 0.24 | 0.19 | 0.20 | 0.19 | Asymmetric bending of $CH_3/CH_2$ of proteins |
| 1600 | 0.24 | 0.24 | 0.23 | 0.21 | 0.20 | 0.20 | 0.21 | 0.18 | 0.20 | Asymmetric COO– stretches |
| 1654 | 0.28 | 0.30 | 0.25 | 0.30 | 0.25 | 0.26 | 0.27 | 0.22 | 0.27 | Amide I of α-helical structure of proteins |
| 2850 | 0.12 | 0.12 | 0.13 | 0.11 | 0.14 | 0.13 | 0.10 | 0.12 | 0.10 | Symmetric stretching of C–H in fatty acids |
| 2855 | 0.13 | 0.12 | 0.13 | 0.11 | 0.14 | 0.13 | 0.10 | 0.12 | 0.10 | C–H of $CH_2$ in fatty acid chains |
| 2870 | 0.13 | 0.13 | 0.14 | 0.12 | 0.15 | 0.15 | 0.11 | 0.14 | 0.11 | Symmetric stretching of $CH_3$ of methyl |
| 2918 | 0.16 | 0.15 | 0.16 | 0.14 | 0.20 | 0.19 | 0.13 | 0.20 | 0.13 | $CH_2$ stretching of methylene in fatty acids |
| 2929 | 0.16 | 0.15 | 0.17 | 0.15 | 0.21 | 0.19 | 0.14 | 0.21 | 0.14 | Asymmetric stretching of C–H and $CH_2$ |
| 2960 | 0.16 | 0.15 | 0.17 | 0.14 | 0.22 | 0.20 | 0.14 | 0.24 | 0.14 | C–H of $–CH_3$ in fatty acids |

[a] WT-SA, WT-ST, WT-KP, OI-SA, CI-ST, CI-KP, CA-SA, CA-ST, and CA-KP denote wild-type *S. aureus*, *S.* Typhimurium, *K. pneumoniae*, oxacillin-induced *S. aureus*, ciprofloxacin-induced *S.* Typhimurium, *K. pneumoniae*, clinically-acquired antibiotic-resistant *S. aureus*, *S.* Typhimurium, and *K. pneumoniae*, respectively

carbohydrate region (1200–900 $cm^{-1}$). No considerable changes in absorbance were observed among WT-SA, OI-SA, and CA-SA, while the distinct peak within the same strains was observed at 1036 $cm^{-1}$ assigned to carbohydrate C–O stretching mode in CI-ST and CI-KP. The change in LPS can plays an important role in the induction of antibiotic resistance in bacteria [22]. The observations suggest that the acquisition of antibiotic resistance in bacteria could lead to the changes in physical properties and chemical components [23]. Although minor changes were not observed in this analysis, FT–IR can be a promising tool to discriminate antibiotic-resistant bacteria based on the major cellular components changes. The spectral features reflect the changes in components specific to antibiotic-resistant bacteria [24].

In conclusion, this study highlights the difference in resistance phenotype of WT-SA, OI-SA, CA-SA, WT-ST, CI-ST, CA-ST, WT-KP, CI-KP, and CA-KP. The phenotypic properties (β-lactamase, efflux pump activity, and cell components) varied with antibiotic susceptibilities of strains tested in this study. The characteristic changes in β-lactam antibiotic susceptibilities were observed after exposure to lactamase inhibitor. However, the susceptibilities of strains to β-lactams were still constant following exposure to tazobactam, implying that the strains tested in this study produced various β-lactamases. In addition, the acquisition of β-lactam resistance is not only attributed to the production of β-lactamases, but also associated with other mechanisms of antibiotic resistance. The characteristic changes in the chemical components of bacteria can be used as biomarkers to detect antibiotic-resistant bacteria. The results would provide useful information regarding resistance phenotypes which is essential for the design of strategies to treat antibiotic-resistant bacterial infection.

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**Compliance with ethical standards**

**Conflict of Interest** There are no conflicts of interest to declare.

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