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RESEARCH ARTICLE

Antagonistic Activities of *Pseudomonas aeruginosa* exoproducts against Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

The rise of Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a serious global health threat and reduces the efficacy of current treatments. The present study investigates the antibacterial and antibiofilm properties of exoproducts from *Pseudomonas aeruginosa* against MRSA. The confirmation of the identities of *P.aeruginosa* and *S.aureus* was achieved through the sequencing of the 16S rRNA gene. Using the agar diffusion assay, the cell-free supernatants (PA-CFS) derived from both the extensively drug-resistant (XDR) *P. aeruginosa* strain and the reference strain ATCC 27853 demonstrated notable antibacterial activity against MRSA, exhibiting inhibition zones ranging from 28.67 ± 0.33 to 33.33 ± 0.33 mm and 26.67 ± 0.33 to 28.66 ± 0.33 mm, respectively. The minimum inhibitory concentration (MIC) assays demonstrated the capability of PA-CFS (XDR) and PA-CFS (ATCC 27853) to suppress MRSA growth at a concentration of 1 × 107 CFU/mL, with stability noted across varied pH and temperature conditions. Biofilm quantification revealed that a 1/2 × MIC of PA-CFS significantly hindered biofilm formation, achieving inhibition rates of 22.301% and 23.886% for PA-CFS (ATCC 27853) and PA-CFS (XDR), respectively. The staphylolytic activity indicated a substantial lysis of MRSA populations influenced by PA-CFS. PCR analysis validated the existence of the *lasA* virulence gene in the two strains of *P. aeruginosa*. GC–MS investigation of ethyl acetate extracts from PA-CFS indicated the presence of 22 distinct peaks for the PA-CFS (ATCC 27853) strain and 8 peaks for the PA-CFS (XDR) strain. These findings indicate that *P. aeruginosa* exoproducts significantly contribute to microbial competition by overcoming challenges posed by MRSA.

Keywords: Antibacterial activity, Exoproduct, GC-MS, MRSA, *Pseudomonas aeruginosa*

Introduction

Bacterial infections from methicillin-resistant *Staphylococcus aureus* (MRSA) pose a huge public health issue due to their resistance to multiple drugs and their potential to cause severe infections.[1] In an effort to combat this issue, researchers have explored the antibacterial activity of various *P. aeruginosa* exoproducts against MRSA. They have found that some of these exoproducts possess antibacterial properties and can effectively hinder the growth of MRSA.[2] These results propose that *P. aeruginosa* exoproducts may hold potential as alternative treatments for MRSA infections. Furthermore, identifying the exact antibacterial components present in these exoproducts and unraveling their mechanisms of action can open up new avenues for drug discovery and the development of effective treatments against MRSA infections.[3] The interaction of various microbial species in burn wound infections and inherited diseases (cystic fibrosis) lung colonization can significantly affect the pathogenicity and drug susceptibility of these microorganisms.[4] Recent findings by Smith et al. indicate that *S. aureus* can endure coculture with *P. aeruginosa in vitro*, specifically in the presence of serum albumin, by inhibiting the *lasR* quorum-sensing system of *P. aeruginosa*, subsequently reducing *LasA* expression.[5]

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Despite these results, *P. aeruginosa LasA*, rhamnolipids, 2-heptyl-4-hydroxyquinoline N-oxide, and pyocyanin are frequently found in CF sputum samples and burn wounds at significant concentrations; they likely have an impact on *S. aureus* physiology.[6] The study suggests that interactions with *P. aeruginosa* may affect the drug susceptibility of *S. aureus, potentially explaining the frequent treatment failures observed in infections involving* drug-susceptible strains. Therefore, this study was designed to explore the *in vitro* antibacterial properties and the efficacy of exoproducts from *P. aeru*ginosa against MRSA biofilms. By exploring the potential of these secreted bioactive compounds, the research aims to identify alternative strategies for combating MRSA infections, which are increasingly resistant to conventional antibiotics. This approach could provide new insights into microbial interactions and open avenues for developing novel antimicrobial agents.

Materials and methods

Bacterial isolation and identification

P. aeruginosa (ATCC 27853) and extensively drug-resistant (XDR) *P. aeruginosa* strains previously isolated from a patient with a burn wound, along with healthcare-associated MRSA, were obtained from the Department of Biology at the University of Sulaimani. These strains were cultivated in Mueller-Hinton broth medium (MHB) (Difco, USA) at 37°C with agitation (225 rpm), while burn wound isolates were grown on Pseudomonas isolation agar base. Identification of the bacterial colonies was performed via biochemical assays, the VITEK®2 system, and confirmed using 16S ribosomal sequencing using primers from Lee et al. (2008), forward F16SrRNA (5-TGGCTCAGATTGAACGCTGGCGGC-3 and reverse R16SrRNA 5-TACCTTGTTACGACTTCACCCCA-3) for PCR amplification, which can amplify 1500 bp.[7] The antibiotic susceptibility of the XDR-*P. The aeruginosa* isolate was assessed with the VITEK 2 system according to the manufacturer's guidelines, and MRSA detection was performed using cefoxitin (30μg) disk diffusion testing.

Preparation of P. aeruginosa cell-free supernatant (PA-CFS)

P. aeruginosa was inoculated in MHB at 37°C with agitation (225 rpm) overnight and adjusted to 106 CFU/mL. The culture was centrifuged at 6,000 g/4°C for 10 min, and the supernatant was decanted away from the pellet and then transferred via a 0.22 μm filter. An aliquot of the PA-CFS was cultured on solid nutrient agar and incubated at 37° C for 48 h to confirm the lack of viable bacteria. Subsequently, the cell-free supernatant was used freshly for further assays.[8]

Agar well diffusion assay

The agar well diffusion technique was utilized to assess the inhibitory effects of PA-CFS on MRSA, following the method adapted from Cardozo et al.[9] Initially, MHA plates were inoculated with a 0.5 McFarland standard of MRSA, after which wells were created using a sterile 8 mm cork borer, into which 100μL of PA-CFS was added. The agar plates were then incubated at 37°C in an aerobic environment, with sterile MHB included as a negative control. The tests were conducted in triplicate, and the effectiveness of PA-CFS was measured by the diameter of the inhibition zones in millimeters at 8, 10, 12, 14, and 16 hours of incubation.

Minimum inhibitory concentrations (MICs) assays

The minimum inhibitory concentrations (MICs) of PA-CFS were assessed using a twofold serial dilution method. Initially, *P. aeruginosa* was cultivated and collected, and then added to MHB medium at a concentration of 1×10^8 CFU/mL. Subsequently, these cultures were diluted to several concentrations, including 5×10^7, 1×10^7, 5×10^6, 1×10^6, 5×10^5, and 1×10^5 CFU/mL. The samples were centrifuged, and the culture supernatants were filtered through a 0.22-μm membrane filter to obtain cell-free supernatants at varying *P. aeruginosa* concentrations. An equal volume (100 μL) of a *S. aureus* bacterial suspension (5×10^5 CFU/mL) was transferred to a 96-well plate containing various concentrations of PA-CFS. The incubation was conducted at 37°C for 24 hrs, and MIC values were identified as the lowest PA-CFS concentration capable of inhibiting observable growth of the pathogenic bacteria.[3,10]

Influence of temperature and acidity on pH antibacterial activity

To evaluate thermostability, the PA-CFS was heated at 60, 80, and 100 °C for 30 min. In parallel, to find out the effect of pH on PA-CFS antibacterial activity, it was incubated at different levels, 5.0, 6.0, 7.0, 8.0 and 9.0 for 30 min. To reduce pH, hydrochloric acid was used, and to increase it, a 1 M NaOH solution was added. The colony counting method was utilized to assess the antibacterial efficacy of the various heat and acid-treated PA-CFS. Precisely, the cell suspensions (MRSA) were adjusted to 5×10^5 CFU/mL. Thereafter, 250 μL of MRSA was mixed with 250 μL

of the treated CFS and transferred to 1.5mL of sterile physiological saline solution. The control group consisted of 250 μL of microbial MRSA suspension mixed with 250 μL of MHB medium and 1.5 mL of sterile physiological saline solution. This mixture was incubated at 37°C for 24 hrs, after which it was diluted and streaked on solid MHA medium. The agar plates were further incubated at 37°C for 24 hours, allowing for the determination of CFU/mL

Biofilm development inhibition assay

To assess the biofilm-inhibiting efficacy of PA (ATCC 27853) and PA-XDR-CFS on MRSA, a series of experiments was conducted. The MRSA suspension was treated with PA-CFS at half the minimum inhibitory concentration (1/2 ×MIC).[11] Following centrifugation, the pellet was rinsed thrice with phosphate-buffered saline and resuspended in tryptic soy broth with 0.5% glucose, adjusting to an absorbance of 0.5 (OD600nm). Each bacterial solution (100 μL) was added to a 96-well plate along with PA-CFS (100 μL) and incubated at 37°C for 24 hrs. A negative control consisted of TSB + 0.5% glucose medium without bacterial suspension, while a positive control contained only the bacterial culture suspension without PA-CFS. Biofilm formation was measured utilizing the crystal violet assay described by Ball et al. (2022. The biofilm was fixed with 205 μL of 100% ethanol per well and allowed to dry. After drying, 205 μL of 1% crystal violet in ddH2O was added, followed by a 15-minute incubation at room temperature. Excess dye was discarded, the wells were washed with 210 μL of ddH2O, and the plate was dried again. Subsequently, 200 μL of 95% ethanol was added, and 100 μL of the destaining solution was transferred to a novel plate for absorbance measurement at 595 nm using a BioTek ELx800 microplate reader. The biomass formation inhibition percentage was calculated using the formula: Percentage inhibition = 100 − [(OD595nm experimental well with PA-CFS/OD595nm control well without PA-CFS) × 100].[12] The microtiter plate biofilm inhibition assay estimates the percentage reduction of MRSA adhesion relative to the control wells, which was 0% in the absence of PA-CFS. This analysis was performed in triplicate, and the average optical density was recorded.

Detection of the mecA gene and LasA by the PCR technique

The detection of the *mecA* and *LasA* genes was conducted using a standard PCR assay with a cycler. DNA was extracted from MRSA and *P. aeruginosa* colonies cultured on Nutrient agar plates, following the protocol of a DNA Extraction Kit from Bioneer Co., Korea. Specific primer pairs were utilized for amplifying 162 bp fragments of the *mecA* gene (**F:** 5”- TCCAGATTACAACTTCACCAGG-3”, and **R:** 5’-CCACTTCATATCTTGTAACG-3‘)[13] and 226 bp fragments of the *LasA* gene (**F:** 5’-CGACAAGAGCGAATACCTGGAG-3”, and **R:** 5”-CAACTG GTATTCCTCGAAACCGTA- 3’).[14] The PCR mixture contained 5 μL of prepared DNA template, 12.5 μL EasyTaq®PCR Super-Mix, 1 μL of 10 pmol/L of each primer, and 5.5μL of sterile distilled water. The thermal cycling process began with a denaturation phase at 94 ˚C for 3 min, followed by 30 cycles of denaturation at 94 ˚C for 30 s, annealing at 54 ˚C and 55 ˚C for 30 s (*mecA* and *LasA* gene), and extension at 72 ˚C for 1 min, concluding with a final extension at 72 ˚C for 7 min. The PCR products were subsequently analyzed through 1.5% agarose gel electrophoresis, stained with ethidium bromide.

The LasA Staphylolytic assay

To evaluate the activity of *LasA* protease, a staphylolytic assay was performed following the methodology of Grande et al.[15] MRSA strains were incubated overnight, and then inactivated by heating for 20 minutes at 95°C. Following centrifugation (7,000 g, 5 minutes), pellets were resuspended in 20 mM Tris-HCl (concentration of OD_{595} 0.8–1). *P. aeruginosa* and *Escherichia coli* (Control) isolates were inoculated into Mueller-Hinton broth for 20 hrs., normalized to an optical density of 2.0 at 600 nm, and the supernatants were collected via a 0.2μm filter. Seventeen microliters of supernatant were mixed with 100μL of heat- inactivated MRSA, and measurements at OD_{595} were taken every 10 minutes for one hour. The data presented indicate the average results of biological triplicates.

Extraction of metabolites from bacterial strains

The PA-CFS was processed for secondary metabolites using ethyl acetate in a 1:1 ratio through a separatory funnel. The resulting ethyl acetate coating was gathered and concentrated with a rotary evaporator. The extract was then transferred to a pre-weighed evaporating dish, desiccated at 40°C in an oven until a constant heaviness was achieved, and finally kept at 4°C.[16]

Gas chromatography mass spectrometry (GC/MS)

The extracts from the PA-CFS were analyzed using the GC-MS method. A volume of one microliter of bioactive compounds at a concentration of 50 μg/μl was introduced into a PerkinElmer Clarus 580

GC/MS, equipped with an Elite-5 MS column (MS 5% diphenyl-95% dimethylpolysiloxane column dimensions of 30 × 0.25 μm ID × 0.25 μm DF). The injector was set to a temperature of 250°C, while the ion source temperature was established at 150°C. The electron impact mode was used for detection at 70 eV, utilizing helium as the carrier gas at a column flow of 1 ml/min. The mass spectrum of the bioactive fragments was recorded at 70 electronvolts (eV) over a 0.5-second interval, and the fragmentation occurred between 45 and 450 Da, which corresponds to the mass-to-charge ratio. The separated ions were identified by the TurboMass detector, and the chromatogram signals were captured and amplified by the TurboMass version 6. 1. 0 program. The system initialization involved starting the solvent at zero minutes, with a two-minute delay prior to a 47-minute GC-MS running period. The resulting mass spectra of the bioactive fragments were subsequently analyzed and matched with the NIST GC-MS spectral database.[17]

Statistical analysis

Statistical analysis was performed utilizing XLStat software (Version 2022.3.1, Addinsoft, Paris, France). Significant differences among independent groups were measured using one-way ANOVA and Tukey's multiple range test, with statistical significance denoted by distinct letters ($p \leq 0.05$).

Results and discussion

Bacterial identification

Bacterial strains were initially identified using both conventional methods (based on cultural, morphological, and biochemical tests) and the Vitek 2 automated system. The Sequencing of the partial 16S rRNA gene demonstrated that all bacterial strains exhibited a similarity of at least 98% to closely related species. To investigate the profiles of extensively drug-resistant (XDR) *P. aeruginosa*, the Vitek platform was utilized for antibiotic resistance characterization. The strain demonstrated resistance to various antibiotics, including beta-lactams (such as penicillins, cephalosporins, and carbapenems), co-trimoxazole, quinolones, at least one aminoglycoside (gentamicin), and nitrofurantoin. These findings align with previous studies documenting antibiotic resistance in *P. aeruginosa* isolated from burn wounds.[18] Multidrug-resistant pathogens, especially MRSA, present a serious public health challenge due to their mechanisms of resistance and their virulent nature.[19] Notably, MRSA strains exhibit thickened cell walls, which contribute to their decreased susceptibility to cell wall-active antibiotics.[20] The study found that S. aureus exhibited significant resistance to cefoxitin, indicating a high prevalence of methicillin resistance. Further genotypic testing confirmed the occurrence of the *mecA* gene in the suspected MRSA isolate, thereby verifying its classification as MRSA, Fig. 1.

PA-CFS Antibacterial Activity against MRSA

The antibacterial activity of PA-CFS (ATCC 27853 and XDR) against MRSA is shown in Fig. 2. PA-CFS exhibited strong antibacterial activity toward MRSA. The average diameter of the ZOI at 8 h of incubation, for PA-CFS (XDR) was 33.33 ± 0.33, and for PA-CFS (ATCC 27853) was 28.66 ± 0.33. At 16 hours of incubation, the average zone of inhibition (ZOI) was significantly reduced to 28.67 ± 0.33 mm for PA-CFS (XDR) and 26.67 ± 0.33 mm for PA-CFS

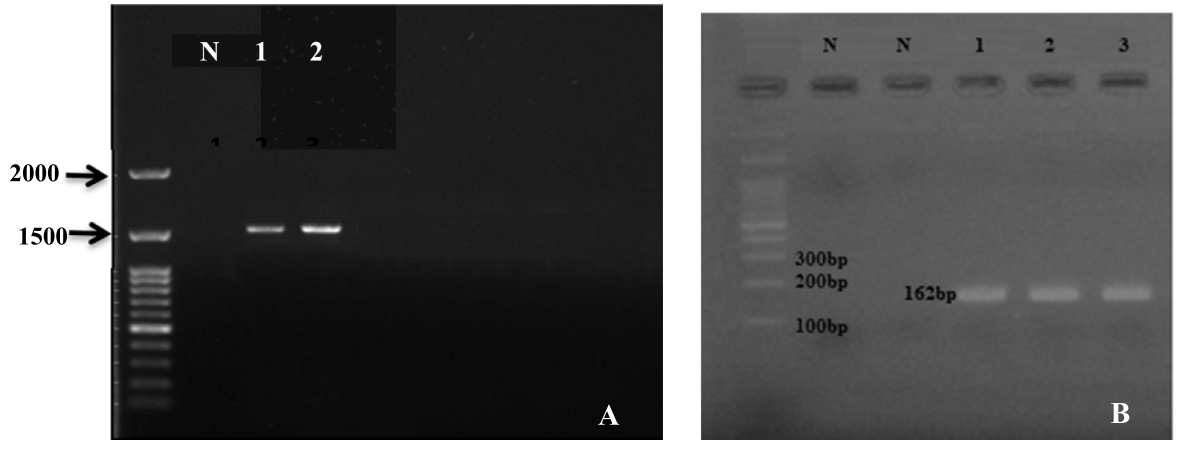

Fig. 1. Gel electrophoresis of PCR product.
A = Lane M: 100 bp DNA ladder, N: represent negative control, lanes 1 & 2 showed positive 1500 bp 16S rRNA gene of *P.aeruginosa*.
B = Lane M: 100 bp DNA ladder, N: represent negative control, lanes 1–3 showed positive 162 bp *mec-A* gene.

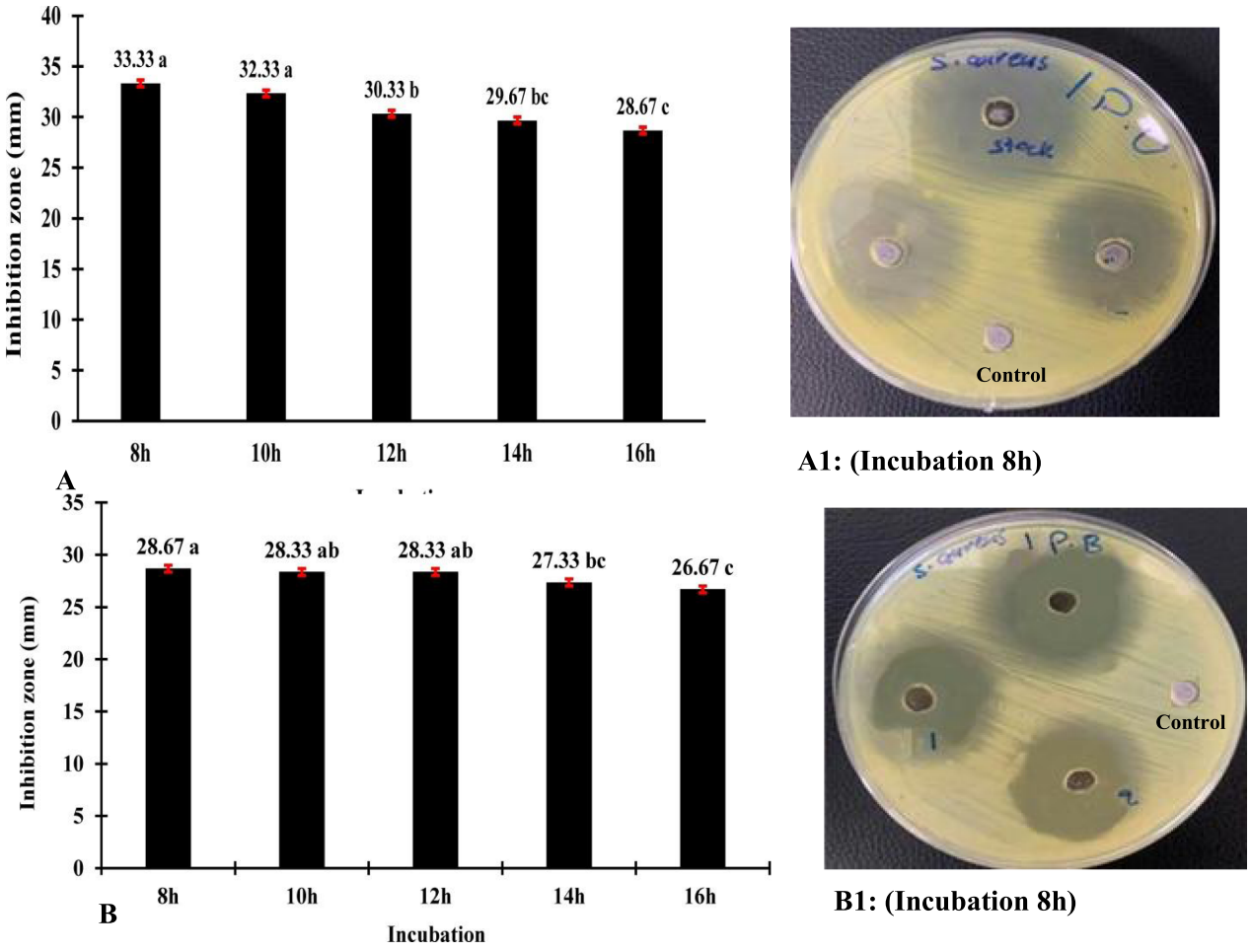

Fig. 2. Antibacterial activity of cell-free supernatant against MRSA: **A** = PA-CFS (XDR) **B** = PA-CFS (ATCC 27853).

(ATCC 27853). The results of MIC assays indicated that PA- CFS (ATCC 27853) and PA-CFS (XDR) can inhibit the growth of MRSA at concentrations of 1 × 10^7CFU/mL. Here, we found that PA-CFS (ATCC 27853 and XDR) had a similarly strong antibacterial effect on MRSA, in agreement with previous studies.[2] *Pseudomonas* species are recognized for their capacity to produce various secondary metabolites, which play significant roles in their virulence and antibiotic properties. Cardozo *et. al.*[9] found that *P. aeruginosa* LV strain produced an organometallic compound with antibacterial activity against MRSA.[17] From a liquid medium of *Pseudomonas* sp. Ki19, dialkylresorcinols were extracted, showing significant inhibition of *S. aureus* at concentrations of 10 g/mL and the fungi *Aspergillus fumigatus* and *Fusarium culmorum* at 50 g/mL.[21] The inhibition zone of selected *Pseudomonas* CFS antimicrobial effects is noted to diminish with time. In this study, the maximum inhibition zone diameter for Pseudomonas CFS was recorded at 8 hours of incubation, followed by a gradual decline. This decrease is attributed to the reduced concentration of the active inhibitory ingredient over time. The results are consistent with earlier research findings.[22]

Influence of temperature and acidity on CFS antibacterial activity

The PA-CFS exhibits stability across a broad spectrum of temperatures between 60–100 ˚C. The PA-CFS (ATCC 27853) reduced the CFU significantly by 5.556 $\log_{10}$, 5.528 $\log_{10}$ and 4.3 $\log_{10}$, while PA-CFS-XDR reduced the CFU by 5.531 $\log_{10}$, 5.505 $\log_{10}$ and 4 log10 for MRSA at 60˚C, 80˚C and 100˚C for 30 min, respectively. The results of the antibacterial activity of PA-CFS against MRSA at different pH levels can be seen in Fig. 3. The PA-CFS produced the highest antibacterial properties against MRSA at pH 7. Meanwhile, the lowest antibacterial activity was shown against MRSA at pH 5 compared to the control. Interestingly, the PA-CFS demonstrated substantial stability in anti-MRSA activity for a wide range of temperatures and pH levels. These findings support previous studies

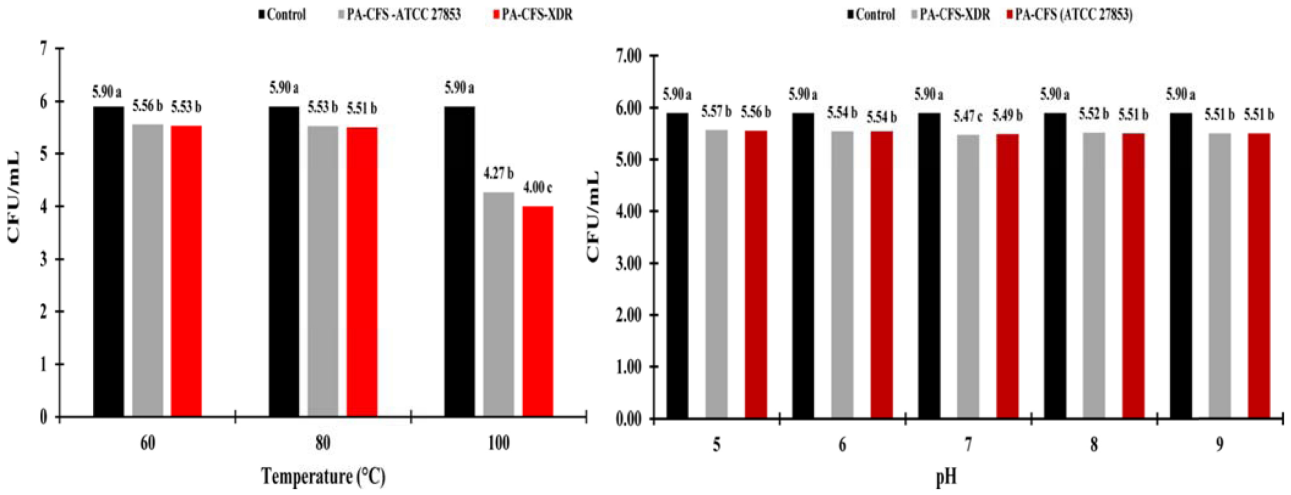

Fig. 3. Reports the CFU values recorded at each time point for each temperature and pH employed.

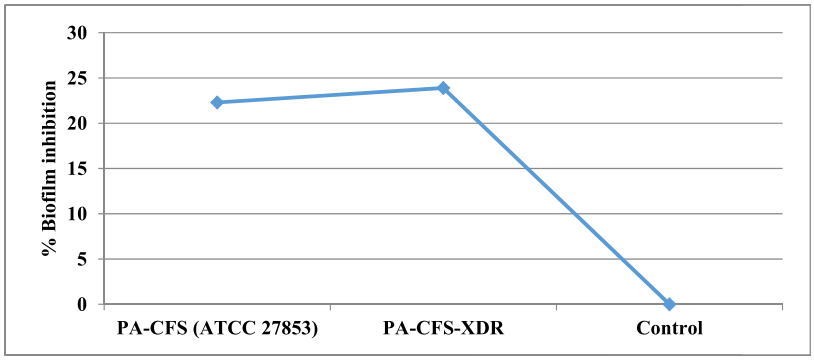

Fig. 4. Effect of various PA-CFS on biofilm formation.

indicating that the supernatant of *P. fluorescens* effectively inhibits biofilm formation of *S. epidermidis* 1457. Importantly, the active compounds responsible for this inhibition remain stable when subjected to temperatures of 100 ˚C for 1–5 minutes, highlighting their potential utility in biofilm control.[23]

According to Charkhian et al.,[24] the antibacterial effects of *P. aeruginosa* on Gram-negative and Gram-positive bacteria can be due to the excretion of bacteriocin molecules of the S-type pyocin. Furthermore, it was discovered that the bacteriocins under study remained stable at temperatures of 50, 70, and 100 ˚C. Although the samples' effectiveness was shown to be somewhat limited by extremely acidic or basic settings (pH: - 2, 12), they were also proven to be robust and maintain their antibacterial qualities throughout a wide pH range.

Reduction of biofilm formation in MRSA by PA-CFS

Quantification of biofilms revealed that the 1/2 × MIC of PA-CFS significantly reduced the amount of biofilm formation in MRSA, Fig. 4. The co-incubation experiment demonstrated that the PA-CFS of ATCC 27853 and PA-CFS-XDR exhibited biofilm inhibition rates of 22.301% and 23.886%, respectively. Our results revealed that PA-CFS has the ability to inhibit biofilm formation by MRSA. In recent years, research has shown that phenazine derivatives produced by *P. aeroginosa* exhibit inhibitory effects on the planktonic form and biofilm formation by MRSA.[16] The compound Pyrrolo (1, 2-a) pyrazine-1, 4-dione, hexahydro-3-(2-methyl propyl), derived from sponge-related aquatic bacteria, was found to inhibit the biofilm development of Loktanella honkongensis and Vibrio halioticoli.[25] The antibiofilm properties of PA-CFS are attributed to its effectiveness in preventing pathogenic bacteria from adhering to biological surfaces. This capacity to inhibit and eliminate bacterial biofilms is associated with the presence of polysaccharides in the CFS. Qin et al. (2009) demonstrated that exoproducts from *P. aeruginosa*, primarily polysaccharides, were capable of disrupting established biofilms of *S. epidermidis*.[26] Moreover, research indicates that *P. aeruginosa* can synthesize cis-2-decenoic acid, an organic compound that promotes the dispersion of established biofilms and inhibits the development of biofilms in

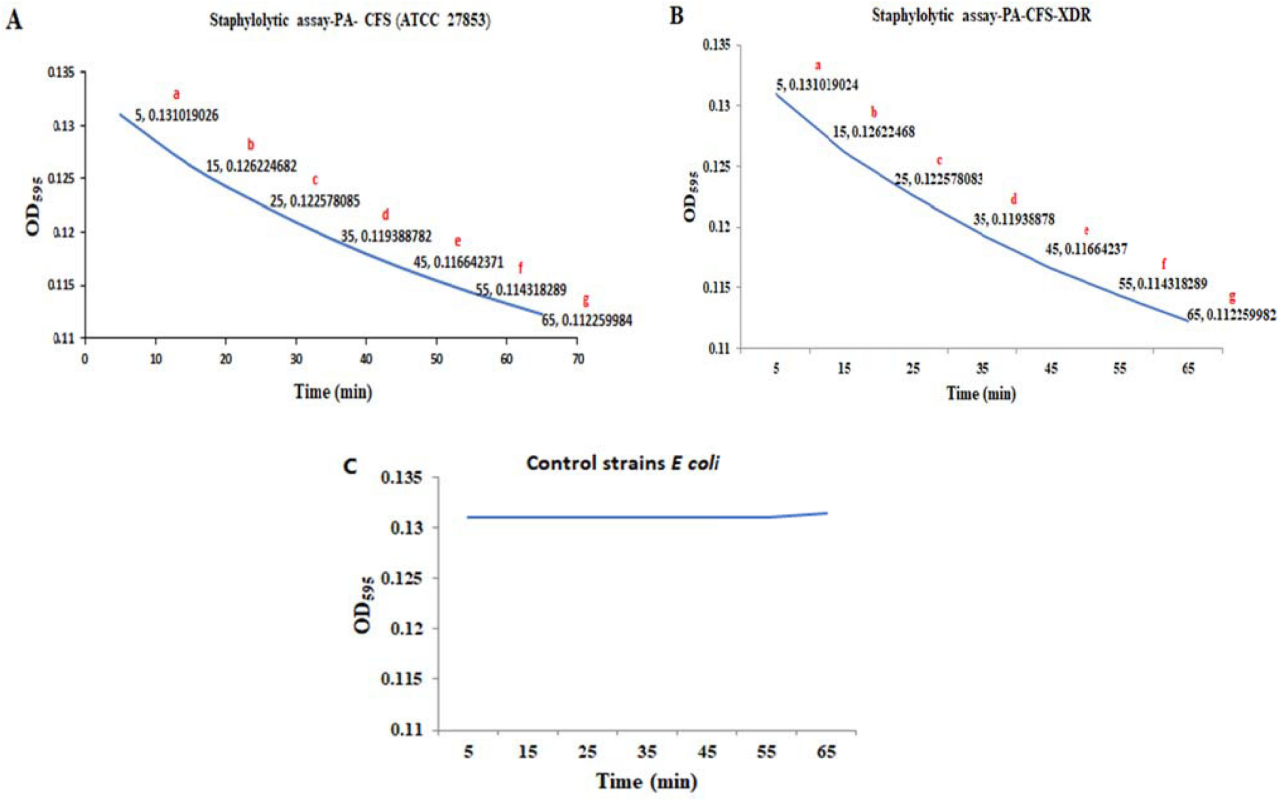

Fig. 5. Staphylolytic activity of cell free supernatant *P. aeruginosa* against MRSA: **A**= PA-CFS (ATCC 27853) **B**= PA-CFS-XDR-CFS **C**= *E. coli* CFS.

various Gram-positive and Gram-negative bacterial species.[27]

The LasA staphylolytic assay

The staphylolytic activity of PA-CFS (ATCC 27853) and PA-CFS-XDR-CFS were examined spectrophotometrically using a heat-killed *S. aureus* suspension. The staphylolytic activity of the *P. aeruginosa* supernatant from the typical strain (ATCC 27853) and clinical XDR demonstrated significant lysis of the bacterial population. The results indicate that extending treatment time from 5 to 65 minutes significantly enhances cell lysis; however, the supernatant of the *E.* coli (control) strain showed no staphylolytic activity Fig. 5. This finding aligns with Radlinsk et al. (2017), who reported a 3-Log reduction in colony-forming units after a vancomycin challenge in the presence of wild-type PAO1, a response which was absent with a PAO1 *lasA* mutant. The *lasA* gene is responsible for cleaving pentaglycine cross-bridges in the peptidoglycan of *S. aureus*, thereby targeting its cell wall during competitive interactions *in vivo*.[28] Antibacterial quinolines can be enclosed in Extracellular membrane vesicles, which induce the rapid disintegration of *S. epidermidis*.[29] An additional extracellular protein released by *P. aeruginosa* that exhibits significant staphylolytic activity is LasA protease,[30] and its expression is regulated by *lasI* and *rhlI*.

LasA protease, also known as staphylolysin, is a 20-kDa endopeptidase released by *P. aeruginosa*.[31] This enzyme effectively lyses various strains of *S. aureus* and has been demonstrated to impede the *in vitro* proliferation of *S. aureus* cells.[32,33]

The PCR results of virulence factor occurrence showed that a *lasA* gene was present in both the PA (ATCC 27853) and PA-XDR strains Fig. 6. Comparison

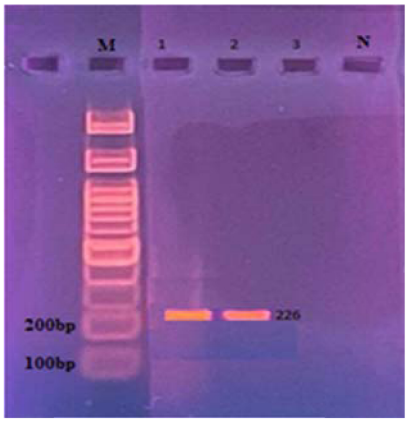

Fig. 6. Gel electrophoresis of PCR product. Lane M: 100bp DNA ladder, N: represent negative control, lanes 1&2 showed positive 226 bp *LasA*- gene.

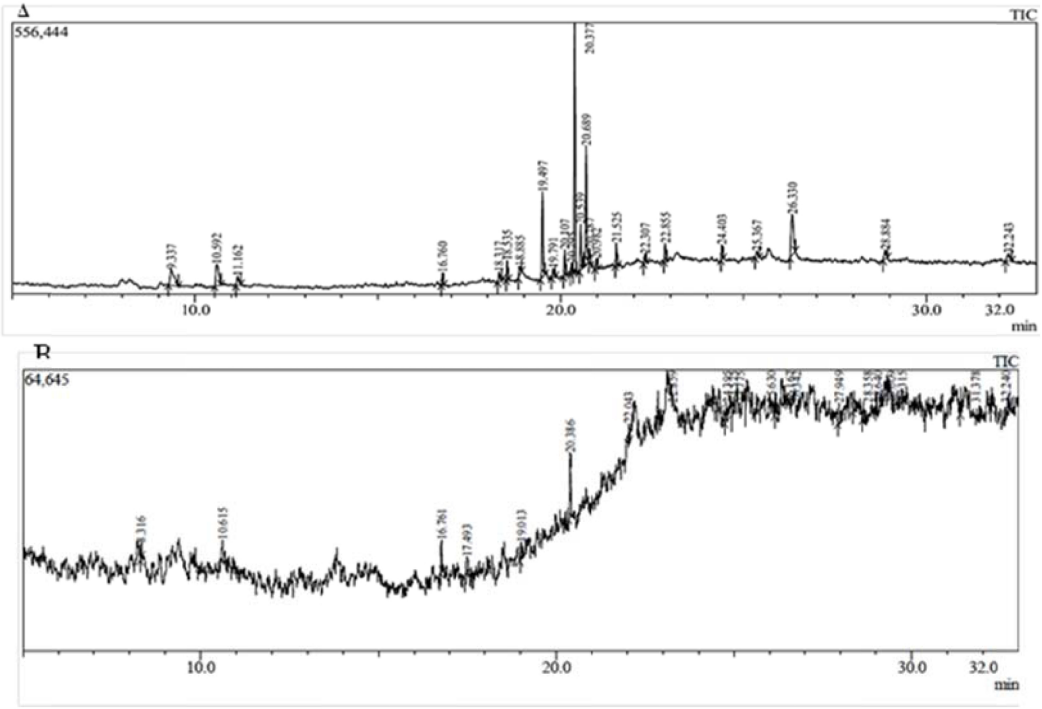

Fig. 7. GC/MS chromatogram of *P. aeruginosa* supernatant: A = (ATCC 27853); B = PA-XDR.

of the *LasA* nucleotide sequence with GenBank records under accession number OP345952 using BLAST software resulted in a complete match with the closest isolate. This aligns with Mahmood's (2023) findings, which indicated that 60% of *P. aeruginosa* isolates harbor these genes.[34] The *LasA* gene is significant as a virulence factor in *P. aeruginosa*, with its expression associated with antibiotic resistance in clinical isolates.[35]

PA-CFS analysis by GC-MS

GC-MS investigation of *P. aeruginosa* strains (ATCC 27853 and XDR) supernatant extracts using ethyl acetate identified 22 and 8 distinct chemical peaks, respectively, according to the NIST database, matching with retention times ranging from 8.31 to 28.88 minutes Fig. 7; Tables 1 and 2. Notably, Benzoic acid, 2, 5_bis (trimethylsiloxy), trimethylsilyl ester was found in both strains.

The study utilized GC-MS to recognize chemical compounds in the ethyl acetate extract of the cell-free supernatant from *P. aeruginosa*, which may contribute to its antagonistic effects against MRSA. Many of these compounds have been documented to exhibit various bioactive properties, including antioxidant, antibacterial, antifungal, and antiviral activities.[36,37] Several authors have identified various compounds with biological activity in ethyl acetate extracts of supernatants from *Pseudomonas* spp., including Pyrrolo (1, 2-a) pyrazine_1, 4-dione, hexahydro compounds,[38] hexadecanoic acid methyl ester, and related benzene derivatives.[37] A GC-MS analysis by Alabi et al.[38] detected compounds such as 1-heptadecanamine, 1-hexanamine, 2_ethyl, 3_butyn-1-ol, Pent_4_enylamine, Propane, Acetaldehyde, Ethylene oxide, Acetonitrile, hydroxy and Pent-3-enylamine in *P. aeruginosa* isolated from poultry fecal litter soil. Notably, these compounds were absent in the PA-CFS (ATCC 27853) and PA-CFS-XDR-CFS extracts, which may be attributed to geographical differences in isolation sources.

The current *in-vitro* analysis indicates that the studied *P. aeruginosa* cell-free supernatants strains (ATCC 27853 and XDR) were more effective as antibacterial agents against MRSA. GC-MS investigation of supernatant extracts from *P. aeruginosa* has identified thirty bioactive chemical components, including Pyrrolo(1,2-a) pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), Pyrrolo (1,2-a) pyrazine-1,4-dione, hexahydro, Pyrrolo(1,2-a) pyrazine-1,4-dione,Heptyl, Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'.alpha.), Sparsomycin, and 2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]. These compounds are likely contributing to the anti-MRSA activity observed in PA-CFS.

Table 1. GC/MS analysis of supernatant ethyl extract of *P. aeruginosa* (ATCC 27853) strain metabolites and a reviewing their previously documented antimicrobial properties.

Peaks NO	RT/min	Bioactive compound name	Mol. formula	Mol. weight	Area: %	Medicinal activity	References
1	9.337	1,3-Butanediol, diacetate	$C_8H_{14}O_4$	174	5.88	Carbapenem synthesis and antioxidant	39,40
2	10.592	2,2-Diethoxytetrahydrofuran	$C_8H_{16}O_3$	160	5.75	Antibacterial and anti-asthmatic	41
3	11.162	1,3-Dioxane, 2-methyl	$C_5H_{10}O_2$	102	1.87	Antitumor	42
4	16.760	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370	1.19	Antimicrobial	43
5	18.317	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*–E)]	$C_{20}H_{40}$	280	1.64	Antibacterial and antiinflammatory	44,45
6	18.535	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	444	1.85	anticancer and antimicrobial	46
7	18.885	Pyrrolo[1,2-a]pyrazine-1,4-dione, Heptyl	$C_7H_{10}N_2O_2$	154	1.21	Antimicrobial and antioxidant	47
8	19.497	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro	$C_7H_{10}N_2O_2$	154	9.72		
9	19.791	3-Pyrrolidin-2-yl-propionic acid	$C_7H_{13}NO_2$	143	1.18	Antimicrobial	48
10	20.107	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	576	2.62	Antioxidants and antimicrobial	49
11	20.295	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.90	Antibacterial, antibiofilm and antioxidant	50,51
12	20.377	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	25.16	Antibacterial and antiparasite	52
13	20.539	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	$C_{11}H_{18}N_2O_2$	210	4.87	Antimicrobial	42,53
14	20.689	Bromocriptine	$C_{32}H_{40}BrN_5O_5$	653	11.87	Antibacterial and antiviral	54,55
15	20.787	Cyclohexaneamine, N-but-2-enylidene-N-oxide	$C_{10}H_{17}NO$	167	1.59	Antioxidant	56
16	20.982	Pentadecane, 1-methoxy-13-methyl-	$C_{17}H_{36}O$	256	1.36	Antioxidants and antimicrobial	57
17	21.525	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	2.31	Antibacterial and anticancer	58
18	22.307	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	466	1.00	Antibacterial	59
19	22.855	1,1,1,5,7,7,7_Heptamethyl-_3,3-bis(trimethylsiloxy) tetrasiloxane	$C_{13}H_{40}O_5Si_6$	444	2.10	Antioxidants and antimicrobial	60
20	24.403	Benzene acetic acid, .alpha.,3,4-tris [(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{20}H_{40}O_5Si_4$	472	1.63	Antibacterial	61
22	26.330	Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'.alpha.)	$C_{33}H_{35}N_5O_5$	581	9.45	Antimicrobial	62
23	28.884	Benzoic acid, 2,6-bis(trimethylsiloxy)-, methyl ester	$C_{14}H_{24}O_4Si_2$	312	1.80	Antibacterial	45

Table 2. GC/MS analysis of supernatant ethyl extract of *P. aeruginosa* (XDR) strain metabolites and a reviewing their previously documented antimicrobial properties.

Peaks NO	RT/min	Bioactive compound name	Mol. formula	Mol. weight	Area: %	Medicinal activity	References
1	8.316	Sparsomycin	$C_{13}H_{19}N_3O_5S_2$	361	2.34	Antiparasitic	63
2	10.615	Benzamide, 3-methyl -N-(7-methyl-2H-[1,2,4]thiadiazolo[2,3-a]pyridin-2-ylidene)-	$C_{15}H_{13}N_3OS$	283	7.93	Anticancer	64
3	16.761	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370	5.29	Antimicrobial	43
4	17.493	Digitoxin	$C_{41}H_{64}O_{13}$	764	4.02	Antimicrobial and anticancer	65,66
6	20.386	Stearic acid hydrazide	$C_{18}H_{38}N_2O$	298	15.85	Antimicrobial	67
7	22.043	Benzoic acid, 5-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	$C_{14}H_{24}O_3Si_2$	296	2.33	Anti-Inflammatory, Antifungal, and Antibacterial	68
8	22.859	1-Propanone, 1,3-diphenyl-3-(trimethylsilyl)	$C_{18}H_{22}OSi$	282	6.00	Antibacterial	69
12	25.630	Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-	$C_{16}H_{28}OSi$	264	2.18	Antibacteria1	70

Conclusion

In conclusion, the cell-free supernatants (PA-CFS) derived from both *Pseudomonas aeruginosa* ATCC 27853 and XDR strains demonstrated significant inhibitory effects on the growth of MRSA. The observed antimicrobial activity was found to be influenced by the duration of incubation, indicating a possible time-dependent effect. GC/MS investigation of the ethyl acetate extracts from these supernatants resulted in the recognition of thirty distinct bioactive compounds. The study indicates that the 1/2 × MIC of PA-CFS significantly decreases biofilm formation in MRSA. Specifically, the co-incubation experiment demonstrated biofilm inhibition rates of 22.301% for ATCC 27853 and 23.886% for PA-CFS-XDR.

The pronounced staphylolytic activity observed in both PA-CFS (ATCC 27853) and PA-CFS-XDR highlights the potential role of the *LasA* protease in disrupting *S. aureus* cell walls, consistent with previous findings. The successful amplification of the *LasA* gene further confirms its presence and supports its involvement as a key virulence factor contributing to the antimicrobial efficacy and resistance traits of *P. aeruginosa* strains.

These findings highlight the potential of *P. aeruginosa*-derived exoproducts as a platform for the discovery of novel anti-staphylococcal agents for infections, especially in the face of rising antibiotic resistance.

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Authors' declaration

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Furthermore, any Figures and images that are not mine have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- Authors signed on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at University of Sulaimani.

References

1. Guo Y, Song G, Sun M, Wang J, Wang Y. Prevalence and Therapies of Antibiotic-Resistance in Staphylococcus aureus. Front Cell Infect Microbiol. 2020;10:107. http://dx.doi.org/10.3389/fcimb.2020.00107.
2. Radlinski L, Rowe SE, Kartchner LB, Maile R, Cairns BA, Vitko NP, *et al.* Pseudomonas aeruginosa exoproducts determine antibiotic efficacy against Staphylococcus aureus. PLoS Biol. 2017;15(11):e2003981. http://dx.doi.org/10.1371/journal.pbio.2003981.
3. Mao Y, Wang Y, Luo X, Chen X, Wang G. Impact of cell-free supernatant of lactic acid bacteria on Staphylococcus aureus biofilm and its metabolites. Front Vet Sci. 2023;10:1184989. http://dx.doi.org/10.3389/fvets.2023.1184989.
4. Reece E, Bettio PH de A, Renwick J. Polymicrobial interactions in the cystic fibrosis airway microbiome impact the antimicrobial susceptibility of Pseudomonas aeruginosa. Antibiotics (Basel). 2021;10(7):827. http://dx.doi.org/10.3390/antibiotics10070827.
5. Alford MA, Mann S, Akhoundsadegh N, Hancock REW. Competition between Pseudomonas aeruginosa and Staphylococcus aureus is dependent on intercellular signaling and regulated by the NtrBC two-component system. Sci Rep. 2022;12(1):9027. http://dx.doi.org/10.1038/s41598-022-12650-2.
6. O'Brien S, Williams D, Fothergill JL, Paterson S, Winstanley C, Brockhurst MA. High virulence sub-populations in Pseudomonas aeruginosa long-term cystic fibrosis airway infections. BMC Microbiol. 2017;17(1):30. http://dx.doi.org/10.1186/s12866-017-0941-6.
7. Lee H-W, Koh YM, Kim J, Lee J-C, Lee Y-C, Seol S-Y, *et al.* Capacity of multidrug-resistant clinical isolates of Acinetobacter baumannii to form biofilm and adhere to epithelial cell surfaces. Clin Microbiol Infect. 2008;14(1):49–54. http://dx.doi.org/10.1111/j.1469-0691.2007.01842.x.
8. Al-Barhawee NIK, Al-Rubyee SS. Inhibition of Biofilm Formation in Agrobacterium tumefaciens by Cell-Free Supernatants of Pseudomonas aeruginosa Analyzed by GC-MS. Baghdad Sci J. 2024;21(7):2222–2236. https://doi.org/10.21123/bsj.2023.8692.
9. Cardozo VF, Oliveira AG, Nishio EK, Perugini MRE, Andrade CGTJ, Silveira WD, *et al.* Antibacterial activity of extracellular compounds produced by a Pseudomonas strain against methicillin-resistant Staphylococcus aureus (MRSA) strains. Ann Clin Microbiol Antimicrob. 2013;12(1):12. http://dx.doi.org/10.1186/1476-0711-12-12.
10. Drumond MM, Tapia-Costa AP, Neumann E, Nunes ÁC, Barbosa JW, Kassuha DE, *et al.* Cell-free supernatant of probiotic bacteria exerted antibiofilm and antibacterial activities against Pseudomonas aeruginosa: A novel biotic therapy. Front Pharmacol. 2023;14:1152588. http://dx.doi.org/10.3389/fphar.2023.1152588.
11. Yu HH, Song YJ, Yu H-S, Lee N-K, Paik H-D. Investigating the antimicrobial and antibiofilm effects of cinnamaldehyde against Campylobacter spp. using cell surface characteristics. J Food Sci. 2020;85(1):157–64. http://dx.doi.org/10.1111/1750-3841.14989.
12. Ball AL, Augenstein ED, Wienclaw TM, Richmond BC, Freestone CA, Lewis JM, *et al.* Characterization of Staphylococcus aureus biofilms via crystal violet binding and biochemical composition assays of isolates from hospitals, raw meat, and biofilm-associated gene mutants. Microb Pathog. 2022;167:105554. http://dx.doi.org/10.1016/j.micpath.2022.105554.

13. Ghaznavi-Rad E, Nor Shamsudin M, Sekawi Z, van Belkum A, Neela V. A simplified multiplex PCR assay for fast and easy discrimination of globally distributed staphylococcal cassette chromosome mec types in meticillin-resistant Staphylococcus aureus. J Med Microbiol. 2010;59(10):1135–9. http://dx.doi.org/10.1099/jmm.0.021956-0.
14. Alwohaili DHK, Alaawad HA-R. Detection of Pseudomonas aeruginosa by duplex polymerase chain reaction assay based on exoA and lasA genes. Biochem Cell Arch. 2019;19(2):(3223–3226). https://doi.org/10.35124/bca.2019.19.2.3223.
15. Grande KK, Gustin JK, Kessler E, Ohman DE. Identification of critical residues in the propeptide of LasA protease of Pseudomonas aeruginosa involved in the formation of a stable mature protease. J Bacteriol. 2007;189(11):3960–8. http://dx.doi.org/10.1128/jb.01828-06.
16. Alatawneh N, Meijler MM. Unraveling the Antibacterial and Iron Chelating Activity of N-Oxide Hydroxy-Phenazine natural Products and Synthetic Analogs against Staphylococcus aureus. Isr J Chem. 2023;63(5–6);e202200112. http://dx.doi.org/10.1002/ijch.202200112.
17. Kumar V, Ahluwalia V, Saran S, Kumar J, Patel AK, Singhania RR. Recent developments on solid-state fermentation for production of microbial secondary metabolites: Challenges and solutions. Bioresour Technol. 2021;323(124566):124566. http://dx.doi.org/10.1016/j.biortech.2020.124566.
18. Choi E, Wells B, Mirabella G, Atkins E, Choi S. Anti-biofilm activity of Pseudomonas fluorescens culture supernatants on biofilm formation of Staphylococcus epidermidis 1457. BMC Res Notes. 2022;15(1):370. http://dx.doi.org/10.1186/s13104-022-06257-z.
19. Algammal AM, Hetta HF, Elkelish A, Alkhalifah DHH, Hozzein WN, Batiha GE-S, *et al.* Methicillin-resistant Staphylococcus aureus (MRSA): One health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infect Drug Resist. 2020;13:3255–65. http://dx.doi.org/10.2147/IDR.S272733.
20. Haunreiter VD, Tarnutzer A, Bär J, von Matt M, Hertegonne S, Andreoni F, *et al.* C-di-AMP levels modulate Staphylococcus aureus cell wall thickness, response to oxidative stress, and antibiotic resistance and tolerance. Microbiol Spectr. 2023;11(6):e0278823. http://dx.doi.org/10.1128/spectrum.02788-23.
21. Pohanka A, Levenfors J, Broberg A. Antimicrobial dialkylresorcinols from Pseudomonas sp. Ki19. J Nat Prod. 2006;69(4):654–7. http://dx.doi.org/10.1021/np0600595.
22. Keeratikunakorn K, Kaewchomphunuch T, Kaeoket K, Ngamwongsatit N. Antimicrobial activity of cell free supernatants from probiotics inhibits against pathogenic bacteria isolated from fresh boar semen. Sci Rep. 2023;13(1):5995. https://doi.org/10.1038/s41598-023-33062-w.
23. Teney C, Poupelin J-C, Briot T, Le Bouar M, Fevre C, Brosset S, *et al.* Phage therapy in a burn patient colonized with extensively drug-resistant Pseudomonas aeruginosa responsible for relapsing ventilator-associated pneumonia and bacteremia. Viruses. 2024;16(7):1080. http://dx.doi.org/10.3390/v16071080.
24. Charkhian H, Soleimannezhadbari E, Bodaqlouei A, Lotfollahi L, Lotfi H, Yousefi N, *et al.* Assessment of bacteriocin production by clinical Pseudomonas aeruginosa isolates and their potential as therapeutic agents. Microb Cell Fact. 2024;23(1):175. http://dx.doi.org/10.1186/s12934-024-02450-w.
25. Dash S, Jin C, Lee OO, Xu Y, Qian P-Y. Antibacterial and antilarval-settlement potential and metabolite profiles of novel sponge-associated marine bacteria. J Ind Microbiol Biotechnol. 2009;36(8):1047–56. http://dx.doi.org/10.1007/s10295-009-0588-x.
26. Qin Z, Yang L, Qu D, Molin S, Tolker-Nielsen T. Pseudomonas aeruginosa extracellular products inhibit staphylococcal growth, and disrupt established biofilms produced by Staphylococcus epidermidis. Microbiology. 2009;155(Pt 7):2148–56. http://dx.doi.org/10.1099/mic.0.028001-0.
27. Davies DG, Marques CNH. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J Bacteriol. 2009;191(5):1393–403. http://dx.doi.org/10.1128/JB.01214-08.
28. Razew A, Laguri C, Vallet A, Bougault C, Kaus-Drobek M, Sabala I, *et al.* Staphylococcus aureus sacculus mediates activities of M23 hydrolases. Nat Commun. 2023;14(1):6706. https://doi.org/10.1038/s41467-023-42506-w.
29. Mashburn LM, Whiteley M. Membrane vesicles traffic signals and facilitate group activities in a prokaryote. Nature. 2005;437(7057):422–5. http://dx.doi.org/10.1038/nature03925.
30. Senturk S, Ulusoy S, Bosgelmez-Tinaz G, Yagci A. Quorum sensing and virulence of Pseudomonas aeruginosa during urinary tract infections. J Infect Dev Ctries. 2012 6(6):501–7. http://dx.doi.org/10.3855/jidc.2543.
31. Kessler E. The secreted aminopeptidase of Pseudomonas aeruginosa (PaAP). Int J Mol Sci. 2024;25(15):8444. http://dx.doi.org/10.3390/ijms25158444.
32. Brito N, Falcón MA, Carnicero A, Gutiérrez-Navarro AM, Mansito TB. Purification and peptidase activity of a bacteriolytic extracellular enzyme from Pseudomonas aeruginosa. Res Microbiol. 1989;140(2):125–37. http://dx.doi.org/10.1016/0923-2508(89)90046-6.
33. Biswas L, Götz F. Molecular mechanisms of Staphylococcus and Pseudomonas interactions in cystic fibrosis. Front Cell Infect Microbiol. 2021;11:824042. http://dx.doi.org/10.3389/fcimb.2021.824042.
34. Mahmood AR. Molecular Analysis of lasA, lasB, and toxA Genes in Clinical Isolates of Pseudomonas Aeruginosa by the PCR Technique. J Nat Sci Biol Med. 2023;14(1):10–6. https://doi.org/10.4103/jnsbm.JNSBM_14_1_2.
35. Jurado-Martín I, Sainz-Mejías M, McClean S. Pseudomonas aeruginosa: An audacious pathogen with an adaptable arsenal of virulence factors. Int J Mol Sci. 2021;22(6):3128. http://dx.doi.org/10.3390/ijms22063128.
36. Amankwah FKD, Gbedema SY, Boakye YD, Bayor MT, Boamah VE. Antimicrobial Potential of Extract from a Pseudomonas aeruginosa Isolate. Scientifica (Cairo). 2022;2022:4230397. http://dx.doi.org/10.1155/2022/4230397.
37. Velusamy P, Jeyanthi V, Pachaiappan R, Anbu P, Gopinath SCB. Secretion of 2,4 di-tertbutylphenol by a new Pseudomonas strain SBMCH11: A tert-butyl substituted phenolic compound displayed antibacterial efficacy. Results Chem. 2024;8(101593):101593. http://dx.doi.org/10.1016/j.rechem.2024.101593.
38. Alabi O. S. Koleoso O B, Abiala A M. Antimicrobial screening and GC-MS analysis of bioactive compounds from strains of Pseudomonas aeruginosa isolated from poultry fecal littered soil in Ibadan, Nigeria. Nig J Pure & Appl Sci. 2019;32(1):3347–3357. https://doi.org/10.6084/m9.figshare.12278864.
39. Maina EG, Madivoli ES, Ouma JA, Ogilo JK, Kenya JM. Evaluation of nutritional value of Asystasia mysorensis and Sesamum angustifolia and their potential contribution to human health. Food Sci Nutr. 2019;7(6):2176–85. http://dx.doi.org/10.1002/fsn3.1064.

40. Matsuyama A, Kobayashi Y, Ohnishi H. Microbial production of optically active 1,3-butanediol from 4-hydroxy-2-butanone. Biosci Biotechnol Biochem. 1993;57(2):348–9. http://dx.doi.org/10.1271/bbb.57.348.
41. Poyil MM, Karrar Alsharif MH, Seshadri VD. Anti-asthmatic activity of Saudi herbal composites from plants Bacopa monnieri and Euphorbia hirta on Guinea pigs. Green Process Synth. 2022;11(1):512–25. http://dx.doi.org/10.1515/gps-2022-0039.
42. Jaber H, Al-Hamaideh K, Al-Daghistani H, Amer N, Nassar M, Al – Latif SMHA, *et al.* Antibacterial activity and chemical composition of arum hygrophilum Boiss crude extracts. Jordan J Biol Sci. 2020;13(2):159. https://doi.org/10.54319/jjbs/170320.
43. Lingfa L, Ankanagari S. GC-MS Profiling of Reproductive Stage Withania somnifera for Antimicrobial and Anticancer Phytochemicals. Biomed Pharmacol J. 2023;16(1). https://dx.doi.org/10.13005/bpj/2601.
44. Singh N, Chhangani N, Bissa S. Antibacterial potential and phytochemical analysis of two ethnomedicinally important plants. Curr Res Microb Sci. 2025;8(100297):100297. http://dx.doi.org/10.1016/j.crmicr.2024.100297.
45. Rahamouz-Haghighi S, Bagheri K, Sharafi A. Antibacterial activities and chemical compounds of Plantago lanceolata (ribwort plantain) and Plantago major (broadleaf plantain) leaf extracts. Pharm Biomed Res. 2023;9(3):183–200. http://dx.doi.org/10.32598/PBR.9.3.1061.4.
46. Lingfa L, Tirumala A, Ankanagari S. GC-MS profiling of anticancer and antimicrobial phytochemicals in the vegetative leaf, root, and stem of Withania somnifera (L.) Dunal. Int J Second Metab. 2024;11(1):63–77. http://dx.doi.org/10.21448/ijsm.1256932.
47. Hallaj-Nezhadi S, Hamdipour R, Shahrvirani M, Zare Tin R, Chapeland-Leclerc F, Ruprich-Robert G, *et al.* Antimicrobial activity of Bacillus sp. isolated strains of wild honey. BMC Complement Med Ther. 2022;22(1):78. https://d-nb.info/1259627837/34.
48. Budayatin, Waluyo J, Wahyuni D, Dafik. Antibacterial effects of Pheretima javanica extract and bioactive chemical analysis using Gas Chromatography Mass Spectrum. J Phys Conf Ser. 2021;1751(1):012055. http://dx.doi.org/10.1088/1742-6596/1751/1/012055.
49. Singh N, Mansoori A, Jiwani G, Solanke AU, Thakur TK, Kumar R, *et al.* Antioxidant and antimicrobial study of Schefflera vinosa leaves crude extracts against rice pathogens. Arab J Chem. 2021;14(7):103243. http://dx.doi.org/10.1016/j.arabjc.2021.103243.
50. Shaaban MT, Ghaly MF, Fahmi SM. Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. J Basic Microbiol. 2021;61(6):557–68. http://dx.doi.org/10.1002/jobm.202100061.
51. A. Ghareeb M, A.H. Hamdi S, Fathi Fol M, M. Ibrahim A. Chemical characterization, antibacterial, antibiofilm, and antioxidant activities of the methanolic extract of Paratapes undulatus clams (Born, 1778). J Appl Pharm Sci. 2022. http://dx.doi.org/10.7324/japs.2022.120521.
52. Kalweit C, Berger S, Kämpfe A, Rapp T. Quantification and stability assessment of 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione leaching from cross-linked polyethylene pipes using gas and liquid chromatography. Water Res. 2023;243(120306):120306. http://dx.doi.org/10.1016/j.watres.2023.120306.
53. Al-Askar, A, Al-Otibi, F, Abo-Zaid, G, Abdelkhalek, A. Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), as the primary secondary metabolite of Bacillus spp., could be an effective antifungal agent against the soil-borne fungus, Sclerotium bataticola. Egyp J Chem. 2024;67(13):1009–1022. http://dx.doi.org/10.21608/EJCHEM.2024.325664.10571.
54. Oselusi SO, Christoffels A, Egieyeh SA. Cheminformatic characterization of natural antimicrobial products for the development of new lead compounds. Molecules. 2021;26(13):3970. http://dx.doi.org/10.3390/molecules26133970.
55. Naz F, Malik A, Riaz M, Mahmood Q, Mehmood MH, Rasool G, *et al.* Bromocriptine therapy: Review of mechanism of action, safety and tolerability. Clin Exp Pharmacol Physiol. 2022;49(8):903–22. http://dx.doi.org/10.1111/1440-1681.13678.
56. Xi P-X, Xu Z-H, Liu X-H, Chen F-J, Huang L, Zeng Z-Z. Synthesis, characterization, antioxidant activity, and DNA-binding studies of 1-cyclohexyl-3-tosylurea and its Nd(III), Eu(III) complexes. Chem Pharm Bull (Tokyo). 2008;56(4):541–6. http://dx.doi.org/10.1248/cpb.56.541.
57. Kumari M, Taritla S, Sharma A, Jayabaskaran C. Antiproliferative and Antioxidative Bioactive Compounds in Extracts of Marine-Derived Endophytic Fungus Talaromyces purpureogenus. Front Microbiol. 2018;9:1777. http://dx.doi.org/10.3389/fmicb.2018.01777.
58. Sultana S, Makeen HA, Alhazmi HA, Mohan S, Al Bratty M, Najmi A, *et al.* Bioactive principles, antibacterial and anticancer properties of Artemisia arborescens L. Not Bot Horti Agrobot Cluj Napoca. 2023;51(1):13008. http://dx.doi.org/10.15835/nbha51113008.
59. Kumari N, Menghani E. Evaluation of antibacterial activity and identification of bioactive metabolites by GCMS technique from Rhizospheric Actinomycetes. Indian J Nat Prod Resour. 2020,12(4);33328. http://dx.doi.org/10.56042/ijnpr.v12i4.33328.
60. Sharma S, Kumar V, Yaseen M, S Abouzied A, Arshad A, Bhat MA, *et al.* Phytochemical analysis, in vitro biological activities, and computer-aided analysis of Potentilla nepalensis Hook compounds as potential melanoma inhibitors based on molecular docking, MD simulations, and ADMET. Molecules. 2023;28(13). http://dx.doi.org/10.3390/molecules28135108.
61. Rahamouz-Haghighi S, Bagheri K, Mohsen-Pour N, Sharafi A. In vitro evaluation of cytotoxicity and antibacterial activities of ribwort plantain (Plantago lanceolata L.) root fractions and phytochemical analysis by gas chromatography-mass spectrometry. Arch Razi Inst. 2022;77(6):2131–43. http://dx.doi.org/10.22092/ARI.2022.358045.2143.
62. Ozma MA, Abbasi A, Sabahi S. Characterization of postbiotics derived from Lactobacillus paracasei ATCC 55544 and its application in Malva sylvestris seed mucilage edible coating to the improvement of the microbiological, and sensory properties of lamb meat during storage. Biointerface Res Appl Chem. 2022;13(3):267. https://doi.org/10.33263/BRIAC133.267.
63. Ariefta NR, Pagmadulam B, Nihei C-I, Nishikawa Y. Sparsomycin exhibits potent antiplasmodial activity in vitro and in vivo. Pharmaceutics. 2022;14(3):544. http://dx.doi.org/10.3390/pharmaceutics14030544.
64. Aliabadi A, Mohammadi-Farani A, Roodabeh S, Ahmadi F. Synthesis and biological evaluation of N-(5-(pyridin-2-yl)-1,3,4-thiadiazol-2-yl)benzamide derivatives as lipoxygenase inhibitor with potential anticancer activity. Iran J Pharm Res. 2017;16(1):165–172. https://doi.org/10.22037/ijpr.2017.1959.
65. Ramasubbu R. GC/MS Analysis of phytoconstituents and antimicrobial evaluation leaf extract of Garcinia

imberti Bourd. IAJMAP. 2024;9(3):134–59. https://doi.org/10.48347/IMIST.PRSM/ajmap-v9i3.49169.
66. Mi C, Cao X, Ma K, Wei M, Xu W, Lin Y, *et al.* Digitoxin promotes apoptosis and inhibits proliferation and migration by reducing HIF-1α and STAT3 in KRAS mutant human colon cancer cells. Chem Biol Interact. 2022;351(109729):109729. http://dx.doi.org/10.1016/j.cbi.2021.109729.
67. Kontham V, Ansari KR, Padmaja KV, Madhu D. Synthesis and evaluation of stearic acid based heterocyclic Schiff bases as biolubricant additives in epoxy karanja fatty acid 2-ethyl hexyl esters base oil. Ind Crops Prod. 2021;159(113061):113061. http://dx.doi.org/10.1016/j.indcrop.2020.113061.
68. Amrati FE-Z, Bourhia M, Saghrouchni H, Slighoua M, Grafov A, Ullah R, *et al.* Caralluma europaea (Guss.) N.e.br. Anti-inflammatory, antifungal, and antibacterial activities against nosocomial antibiotic-resistant microbes of chemically characterized fractions. Molecules. 2021;26(3):636. http://dx.doi.org/10.3390/molecules26030636.
69. Soltani E-K, Mokhnache K, Mezaache-Aichour S, Charef N, Haro JPD, Zerroug MM, *et al.* Chemical composition and antibacterial activity of Algerian propolis against fish pathogenic bacteria. J Drug Deliv Ther. 2020;10(2):12–9. http://dx.doi.org/10.22270/jddt.v10i2.3904.
70. Salih AM, Qahtan AA, Al-Qurainy F. Phytochemicals identification and bioactive compounds estimation of Artemisia species grown in Saudia Arabia. Metabolites. 2023;13(3). http://dx.doi.org/10.3390/metabo13030443.

الأنشطة المضادة لمنتجات بكتيريا *Pseudomonas aeruginosa* ضد المكورات العنقودية الذهبية المقاومة للميثيسيلين

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الخلاصة

ظهور المكورات العنقودية الذهبية المقاومة للميثيسيلين(MRSA) يشكل تهديدًا خطيرًا للصحة العالمية ويقلل من فعالية العلاجات الحالية. في هذا البحث ، تم تقييم الخصائص المضادة للبكتيريا والغشاء الحيوي للمنتجات الخارجية من Pseudomonas aeruginosa ضد MRSA أكد تسلسل جين 16S rRNA تشخيص P. aeruginosa و S.aureus. باستخدام طريقة الانتشار على الأجار، أظهر الراشح الخالي من خلايا P. aeruginosa كل من PA-CFS (XDR) و PA-CFS (ATCC 27853) تأثيرات مضادة ضد MRSA، مع قياس مناطق التثبيط بين33.33±0.33- 28.67±0.33 ملم و 26.67±0.33 - 28.66 ±0.33 ملم، على التوالي. أظهرت اختبارات التركيز المثبط الأدنى (MIC) قدرة PA-CFS (XDR) و PA-CFS (ATCC 27853) على كبح نمو MRSA عند تركيز 1 × 107 وحدة مكونة للمستعمرات/مل، مع ملاحظة الاستقرار عبر ظروف الأس الهيدروجيني ودرجات الحرارة المختلفة. كشفت كمية الغشاء الحيوي أن 2/1 × MIC من PA-CFS عاق بشكل كبير تكوين الغشاء الحيوي، محققًا معدلات تثبيط بلغت 22.301% و 23.886% لـ PA-CFS (ATCC 27853) و PA-CFS (XDR)، على التوالي. أشارت الفعالية ضد المكورات العنقودية إلى تحلل كبير لمجموعات MRSA تأثرت بـ PA-CFS. علاوة على ذلك، أكد تحليل PCR وجود الجين الممرض lasA في كلا سلالتي P. aeruginosa ، بينما حدد تحليل GC-MS للمستخلص الإيثيلي من محلولها الخالي من الخلايا 22 و 8 قمم تتوافق مع سلالتي PA-CFS (ATCC 27853) و PA-CFS (XDR)، على التوالي، مكشوفًا عن مركبات مثل حمض البنزويك، 2، 5-ثنائي(تريميثيلسيلوكسي)-, إستر ثلاثي ميثيل السيليل. تشير هذه النتائج إلى أن المنتجات الخارجية لبكتريا P. aeruginosa تلعب دورًا كبيرًا في التنافس الميكروبي من خلال التغلب على التحديات سلالات بكتريا .MRSA

الكلمات المفتاحية: النشاط المضاد للبكتيريا، المنتج الخارج ، Pseudomonas aeruginosa, MRSA ، GC-MS