The prevalence and mechanism of triclosan resistance in Escherichia coli isolates from urine samples in Wenzhou, China

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Research

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Abstract

Background

Widespread use of triclosan has been reported to cause its residue in urine, which provides an environment of long-term exposure to triclosan for intestinal *Escherichia coli*. We aimed to determine the triclosan and antibiotic resistance characteristics of *Escherichia coli* strains isolated from urine, and further investigate the resistance mechanism and molecular epidemic characteristics of triclosan resistant *Escherichia coli* isolates.

Methods

A total of 200 non-repetitive *E. coli* strains from urine samples were obtained and identified. The minimum inhibitory concentrations (MICs) of triclosan and antibiotics, *fabI* mutation, efflux pump activity, expression of 14 efflux pump encoding genes and epidemiological characteristics were detected with agar dilution method, polymerase chain reaction (PCR), carbonyl cyanide 3-chlorophenylhydrazone (CCCP) inhibition test, quantitative real-time polymerase chain reaction (RT-qPCR), multilocus sequence typing (MLST) and pulse field gel electrophoresis (PFGE) in all triclosan resistant isolates. Furthermore, we also investigated the effect of triclosan exposure in vitro on resistance in susceptible strains by serial passage experiment.

Results

Of 200 *E. coli* isolates, 2.5% (n = 5) were resistant to triclosan, multidrug resistance (MDR) and cross-resistance phenotypes were observed in these resistant strains, but not in susceptible strains. We did not observe any sense mutations within *fabI* gene in triclosan resistant strains. Moreover, except DC8603, all the others enhanced efflux pumps activity. Compared with ATCC 25922, except *fabI*, increased expression were also found in efflux pump encoding genes *ydcV*, *ydcU*, *ydcS*, *ydcT*, *cysP*, *yihV*, *acrB*, *acrD* and *mdfA* in studied strains with different PFGE patterns and STs types. Surprised, 5 susceptible *E. coli* isolates increased rapidly triclosan resistance only 4 days after exposure to subinhibitory triclosan concentration in vitro.

Conclusions

Our study is the first to be reported that short-term triclosan exposure in vitro increases triclosan resistance in susceptible *E. coli* isolates. Once strains have acquired resistance, they usually present MDR or cross-resistance phenotypes. Besides, our findings indicate that triclosan resistance were mainly involved by *fabI* overexpression in *E. coli*, and there was a close association between overexpression of efflux pumps with triclosan resistance.

Background

Escherichia coli isolates are responsible for the most hospital and community acquired infections, such as a variety of intestinal and extraintestinal infections, urinary tract infection, as well as serious infections in the immunocompromised patients [1–3]. Over the past few decades, self-medication and antibiotics misuse led to the increasing resistance in clinical practice. Even worse, the treatment of infections caused by *E. coli* is challenging because of the increasing multidrug-resistance to antibiotics [4, 5].

Triclosan, a broad-spectrum and highly effective antibacterial agent, can inhibit various microorganisms at low concentrations, and be bactericidal at high concentrations [6]. In fact, it is not only used in disinfections, but also in medical equipment to prevent infections [7]. Hence, triclosan plays a key role in reducing the dissemination and spread of pathogenic bacteria in hospital and community environments.

Unfortunately, owing to increased clinical use, obvious levels of triclosan in various natural and engineered environment, such as soil and water, even in babies and adults fluids, such as urine, have been reported [8–10]. In China, Yin et al. estimated the average concentration of triclosan reached 0.36 μg/l in 80% urine samples [11]. Furthermore, triclosan was known as a “new environmental endocrine disruptor” due to its potential endocrine disrupting effects, which took an adverse effect on human health [12]. To make matters worse, recent study has shown that triclosan can spread antibiotic resistance genes [13]. In short, if long-term use of triclosan is contributing to triclosan residue in human urine, and the impact of the widespread use of triclosan on bacterial resistance has been controversial, both of above will affect our health, then more evidences about the effect of triclosan on resistance in *E. coli* isolates from urine should be reported [14].

Long-term exposure to triclosan have promoted a reduced sensitivity to triclosan in *E. coli* through extensive resistance mechanisms in vitro [15]. Of these resistance mechanisms, active efflux, reducing the drugs concentration in the bacteria whereby efflux pump systems to pump the intracellular antibacterial drugs out of the cell, conferred bacteria the ability to against a wide range of antimicrobials and biocides, including triclosan [16, 17]. Indeed, many drug efflux pumps are known to mediate resistance of traditional antibiotics and biocides, including the resistance nodulation division (RND) family, the major facilitator superfamily (MFS), the staphylococcal multi-resistance (SMR), the multidrug and toxic compound extrusion (MATE) families [18]. Additionally, *fabI* mutation also contributes to *E. coli* resistance to triclosan [19]. However, the role of different type efflux pumps is not well understood in triclosan resistant *E. coli* isolated from urine.

In order to provide a better scientific theoretical basis for the rational use of triclosan and nosocomial infections control, investigations are urgently warranted. Our study described the resistance profile of *E. coli* isolates from urine, and further investigated mechanism of the action of triclosan as well as molecular epidemiological characteristics.

Methods

Bacterial strains and identification

A total of 200 non-repetitive *E. coli* strains from urinary tract infection (UTI) patient urine were obtained from Affiliated Hospital of Wenzhou Medical University in Wenzhou, China in 2018. All bacteria were identified by the Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS; bioMérieux, Lyons, France).

Minimum Inhibitory Concentrations Of Triclosan

We measured the MICs of triclosan according to previously study, isolates with MICs ≥ MIC_{90} (the concentration required to inhibit growth by 90% isolates; MIC_{90} = 0.5 μg/ml) were classified as resistance [20]. *E. coli* ATCC 25922 served as the quality control strain.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was also performed for 10 clinical conventional antibiotics by agar dilution method, and the results were interpreted by the latest guidelines from the Clinical and Laboratory Standards Institute (CLSI).

Detection of fabI gene mutation by PCR

Genome DNA of triclosan resistant *E. coli* strains, as well as randomly selected equal numbers of triclosan susceptible strains, were extracted using the Biospin Bacterial Genomic DNA Extraction kit (Bioflux, Tokyo, Japan) according to the manufacturer's instructions. Then, *fabI* gene and 14 known drug efflux pump encoding genes (*ydcT*, *ydcU*, *ydcV*, *ydcS*, *cysP*, *cysU*, *marA*, *soxS*, *yhiv*, *acrB*, *acrD*, *acrF*,*mdfA* and *norE*) were amplified by PCR with the specific oligonucleotide primers, positive PCR products were directly sequenced by Shanghai Genomics Institute Technology Co. Ltd [17]. Genetic mutations were further analyzed by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi; GenBank accession number: NC000913.3). Primer of *fabI* gene was listed in Supplementary Table S1 (see Additional file 1).

Efflux Pump Inhibition Test

To test efflux pump activity of triclosan resistant *E. coli* strains, efflux pump inhibitor CCCP was trialed. The resistant strains were tested on agar plates with the presence or absence of 10 μg/ml CCCP by the agar dilution method. Compared with triclosan alone, MICs value of triclosan decreased ≥ 4 was confirmed having an inhibitory effect when triclosan was used in combination with 10 μg/ml CCCP [21]. In addition, the 10 μg/ml concentration was determined as the ideal concentration using the agar dilution method.

Expression Levels Of Efflux Pumps By Rt- Qpcr

Except detecting *fabI* expression, 14 efflux pump encoding genes were also checked using RT-qPCR, including the ABC transporters system encoding genes *ydcT*, *ydcU*, *ydcV*, *ydcS*, *cysP* and *cysU*, the Arac-regulator genes *marA* and *soxS*, the RND efflux pump encoding genes *yhiv* and *acrBDF*; the MdfA efflux TolC encoding genes *mdfA*; and the NorE efflux pump encoding gene *norE*.

Briefly, triclosan resistant strains with active efflux pump were included, ATCC 25922 served as the control strain. The strains above were inoculated in fresh Luria broth (LB) and allowed to grow to logarithmic phase (OD_{600} = 0.6). The total cellular RNA of these cultures was extracted using the Bacterial RNA Miniprep Kit (Biomiga, Shanghai, China) according to the manufacturer's recommendation. Subsequently, the purified RNA was subjected to reversely transcribed into cDNA by means of the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, MA, USA), amplification was performed using TB Green Premix Ex Taq II (Tli RNaseH Plus) (2×) (Takara, Japan). In the PCR reaction, a global gene *gapA* and housekeeping gene *16S rRNA* were used as corresponding internal control, the quantification of efflux pump genes was performed by the $2^{-\Delta\Delta Ct}$ method. Compared to the control strain ATCC 25922, an expression ≥ 2 indicated up-regulation according to previous study [22]. Specific RT-qPCR primers were listed in Supplementary Table S1 (see Additional file 1). All experiments were performed in triplicates and the data was displayed as the mean ± SD values in Supplementary Table S2 (see Additional file 1).

Genotyping By Mlst

All the triclosan non-susceptible isolates were typed using MLST method. The sequences of 8 housekeeping genes (*trpB*, *uidA*, *dinB*, *icdA*, *pabB*, *polB*, *put* and *trpA*) were amplified with specific primers available at the MLST database (https://bigsdb.pasteur.fr/index.html), and sequence types (STs) were evaluated by comparing the allelic profiles to the MLST database.

Strain Typing Pfge

To confirm and analyze the clonal relatedness of resistant isolates, PFGE was also used for analysis the clonal relatedness of the triclosan resistant isolates, according to the PulseNet protocols published by the US Centers for Disease Control and Prevention (CDC) with minor modifications. The cell suspensions treated with protease K were incubated with XbaI restriction enzyme at least for 2 hours at 37 ºC to digest the DNA fragments. Then PFGE was performed using a CHEF-MAPPER XA PFG system (Bio-Rad, USA) for 18 hours. The detailed running condition were as follows: initial switch time value of 2.16 sec, final switch time of 54.17 sec at a gradient of 6 V/cm at a 120° included angle [23]. Next, the electrophoretic banding patterns were visualized by GelDoc XR gel imaging system (Bio-Rad, USA) and further analyzed by Quantity One (Bio-Rad Laboratories, USA). The Unweighted Pair Group Method with Arithmatic Mean (UPGMA) with optimization set at 1.5% to create the dendrogram, cut off line ≥ 85% was considered to analyze genetic relatedness [24]. *Salmonella* standard strain H9812 was taken as the positive control.

Serial Passage Experiment

In order to determine whether triclosan exposure in vitro increased bacterial resistance, as previously described, serial passage experiment was conducted for triclosan susceptible isolates DC8361, DC8363, DC8400, DC8413 and DC8510[25]. Specifically, the isolates were cultivated on Maconkey ager plate and cultured overnight at 37 ºC to obtain a single isogenic strain, which was then inoculated into 3 ml fresh LB broth with different concentrations of triclosan at 37 ºC overnight, the ticlosan gradient concentrations were 0.0625, 0.125, 0.25, 1, 2, 4, 8, 16 and 32 μg/ml. Culture supernatants with bacteria growing in the highest triclosan concentrations were aspirated and continuously passaged in new triclosan gradients, after only four days of triclosan exposure, triclosan-mutant strains with MICs ≥ 0.5 μg/ml were obtained.

Next, the stability of triclosan resistance was confirmed by continuous passage in vitro. Briefly, the triclosan-mutant strains were cultured in 3 ml fresh LB broth without triclosan at 37 ºC for 24 hours. Every 24 hours, 30 μl of overnight culture supernatants were transferred to another 5 ml tube containing 2.97 ml fresh LB broth without triclosan. After six cycles, the MICs of triclosan as well as ten antibiotics were tested in triplicate respectively using the same method described previously.

Results

Isolates with reduced susceptibility to triclosan presented MDR or cross-resistance phenotypes

MICs of triclosan among 200 isolates were ranging from 0.03125 μg/ml to 8 μg/ml, 5 triclosan resistant isolates were selected (2.5%, 5/200), as shown in Table 1. there was a low triclosan tolerant rate of *E. coli* from urine specimens. Interestingly, these resistant isolates tend to be resistant to multiple antibacterial agents, including ampicillin, cefepime, ceftazidime and gentamycin, but randomly selected 5 triclosan-sensitive strains did not exhibit these phenotypes. Based on this, we are interested in exploring this issue: Whether triclosan exposure in vitro has the same effect on bacterial resistance? Further serial passage experiment was needed and performed.

Table 1
Mutations of *fabI* gene and MICs of triclosan and antibiotics against *E. coli* strains

Isolates	Triclosan MICs (μg/ml)	Antibiotic MICs [a] (μg/ml)										Mutations in *fabI* [b]
		AMP	CIP	LVX	FEP	CAZ	ETP	IPM	GEN	NIT	TOB	
DC8358	8	**> 128** [c]	0.5	0.5	**32**	**16**	**16**	2	**> 64**	64	**> 64**	ND [d]
DC8419	4	**> 128**	**> 32**	**64**	**16**	**16**	< 0.5	< 0.25	**> 64**	32	**32**	Gly79Ala
DC8424	4	**> 128**	**> 32**	**16**	**> 64**	**> 64**	**> 32**	**> 32**	**> 64**	64	**> 64**	Ala69Thr
DC8603	2	**> 128**	**> 32**	**> 64**	**64**	**32**	< 0.5	< 0.25	< 2	**> 128**	< 2	ND
DC8724	8	**> 128**	**> 32**	**32**	**32**	**64**	**16**	1	**> 64**	16	**> 64**	ND
DC8361	0.125	< 4	< 0.25	0.5	< 1	< 2	< 0.5	< 0.25	< 2	16	< 2	Met2Arg; Val5Phe; Ala69Thr
DC8363	0.125	**> 128**	< 0.25	0.5	2	< 2	< 0.5	< 0.25	< 2	< 8	< 2	Ser5Leu; Gly79Ala
DC8400	0.125	**> 128**	< 0.25	< 0.25	< 1	< 2	< 0.5	< 0.25	< 2	16	< 2	Val4Ser; Ala69Thr
DC8413	0.25	**> 128**	< 0.25	< 0.25	< 1	< 2	< 0.5	< 0.25	< 2	< 8	< 2	Gly79Ala
DC8510	0.125	**> 128**	< 0.25	< 0.25	2	< 2	< 0.5	< 0.25	< 2	< 8	< 2	Gly79Ala; Asp 235Glu

[a] MICs, Minimum inhibitory concentration.

AMP, ampicillin; CIP, ciprofloxacin; LVX, levofloxacin; FEP, cefepime; CAZ, ceftazidime; ETP, ertapenem; IPM, imipenem; GEN, gentamycin; NIT, nitrofurantoin; TOB, tobramycin.

[b] Gly, Glicine; Ala, Alanine; Thr, Threonine; Met, Methionine; Arg, Arginine; Val, Valine; Phe, Phenylalanine; Ser, Serine; Leu, Leucine; Asp, aspartic acid; Glu, glutamic acid.

[c] Bolded values point means resistance.

[d] ND, Not detected.

Derived isolates without MDR or cross-resistance phenotypes rapidly reduced susceptibility to triclosan after short-term triclosan exposure in vitro

Stable triclosan-mutant strains were generated by continuous passage in triclosan susceptible isolates DC8361, DC8363, DC8400, DC8413 and DC8510. the MICs of tested antibiotics were no change in Supplementary Table S3 (see Additional file 1). But more remarkable, after exposure to triclosan with subinhibitory concentration in vitro only 4 days, derived triclosan resistant strains quickly emerged, and the MICs of triclosan increased by 8-128 times.

Analysis of fabI mutation

PCR revealed *fabI* and 14 efflux pump encoding genes were present in all tested strains, except *acrF* gene. A variety of different mutations were detected in both resistant and susceptible strains. In DC8419 and DC8424, the Gly79Ala and Ala69Thr mutations were found, respectively. However, these mutations were also observed in susceptible strains. Besides, we discovered other mutations of *fabI* gene in susceptible strains, such as Met2Arg, Ser5Leu, Val4Ser and Asp235Glu (Table 1).

Efflux Pump Phenotype Test

We sought to further investigate the triclosan resistance mechanism among resistant isolates. Compared to the absence of CCCP, the triclosan MICs of DC8358, DC8419, DC8424 and DC8724 reduced by 8, 4, 4 and 16 times in the presence of 10 μg/ml CCCP respectively. The result indicated that efflux pumps systems were extremely active among above mentioned 4 isolates. Inversely, DC8603, unlike other resistant strains, showed a negative phenotype in efflux pump test, which the MICs of triclosan had not changed whether with or without CCCP (Table 2).

Table 2
Efflux pump phenotype test

Isolates	MICs (μg/ml)		fold changes	Efflux pump phenotype*
	Triclosan	Triclosan + CCCP (10 μg/ml)		
DC8358	8	1	8	+
DC8419	4	1	4	+
DC8424	4	1	4	+
DC8603	2	2	1	-
DC8724	8	0.5	16	+

* Compared with triclosan alone, MICs value of triclosan decreased ≥ 4 was confirmed having an inhibitory effect when triclosan was used in combination with 10 μg/ml CCCP. + means strains with positive efflux pump phenotype. - means strains with negative efflux pump phenotype.

Expression levels of fabI and efflux pump encoding genes

The expression levels of *fabI* were evaluated in this study. Increased expression (> 2-fold) of *fabI* gene was observed in all triclosan resistant strains. Compared with triclosan susceptible control strain ATCC 25922, the fold-changes of *fabI* gene were between 5.69 to 41.85 times (Fig. 1).

In addition, to gain the better understanding of the relationship between triclosan resistance and efflux pump genes expression levels, different efflux pump types were also examined, as shown in Fig. 2 and Supplementary Table S2 (see Additional file 1). Compared to *E. coli* ATCC 25922, the expression of *ydcV* was increased obviously (> 2-fold) in DC8358 (fold-changes: *ydcV*, 5.71 ± 0.68). Exhibited enhanced expressions of *ydcV*, *yihV* and *acrB* (fold-changes: *ydcV*, 8.74 ± 0.61; *yihV*, 3.57 ± 0.52; *acrB*, 3.44 ± 0.21, respectively) were found in DC8419. For DC8424, the expressions of *ydcU*, *ydcS*, *yihV*, *acrD*, and *mdfA* (fold-changes: *ydcU*, 4.71 ± 0.13; *ydcS*, 2.8 ± 0.42; *yihV*, 6.82 ± 0.65; *acrD*, 2.63 ± 0.14; *mdfA*, 5.13 ± 0.26, respectively) were increased. Upregulation of active efflux pump genes *ydcT*, *ydcU*, *ydcS*, *cysP*, and *yihV* (fold-changes: *ydcT*, 6.56 ± 0.56; *ydcU*, 18.25 ± 1.36; *ydcS*, 8.76 ± 0.49; *cysP*, 3.89 ± 0.2; *yihV*, 2.00 ± 0.03, respectively) were observed in DC8724. The results were consistent with efflux pump inhibition test, which indicated that efflux pumps overactivity induced overexpression of efflux pump genes, which in turn mediates triclosan resistance in *E. coli* isolates.

Molecular Epidemiological Analysis

PFGE analysis revealed that the similarity of these isolates was low (< 0.85) due to the large differences in PFGE patterns. Similarly, the results of MLST confirmed that they were categorized into multiple and scattered STs, including ST3, ST833, ST567, ST471 and ST1, respectively (Fig. 3). In short, the results above illustrated that triclosan resistant strains had extremely low clonal relatedness in this study.

Discussion

With the popular use of disinfectants in clinical or household, including triclosan, increasing evidences showed that triclosan were found in human body, such as urine, which caused people's concern. At present, the relationship between biocides and antimicrobial resistance remains still controversial, and the effect of triclosan exposure on bacterial resistance is also unclear.

In our study, 5 triclosan resistant strains (2.5%, 5/200) were selected out of 200 *E. coli* isolates collected from urine samples, we observed the lower resistance rates than the previous report, and these triclosan resistant strains were characterized by MDR profiles [20]. Hence, we ask the following questions: Whether triclosan exposure affects bacterial resistance? Or whether derived resistant isolates exhibit similar phenotypes?

Further research was carried out, we randomly selected 5 strains (DC8361, DC8363, DC8400, DC8413 and DC8510) that were sensitive to triclosan and almost all antibiotics for serial passage experiment, which proved that derive triclosan resistant isolates emerged only 4 days after triclosan exposure in vitro, although these isolates did not present MDR or cross-resistance phenotypes. These results suggested short-term triclosan exposure rapidly increased bacterial resistance to triclosan, but not antimicrobial agents. One possible explanation is that, MDR or cross-resistance phenotypes are related to the exposure time of triclosan. Actually, it has been reported that bacteria develop cross-resistance under the long-term selective pressure of triclosan, rather than short-term, that's why cross-resistance or MDR profiles have not been observed in our study [26, 27].

Based on the obtained phenomena and previous reports, we thought that the emergence or evolution of multiple or cross-resistance is related to the exposure time of triclosan, which provided a sufficient evidence for clinical practice to avoid long-term use of triclosan [22, 26]. Moreover, it is worth noting that significantly reduced triclosan sensitivity was found by serial passage experiment, which provided a beneficial guidance for reasonable use of triclosan, including concentration and dosage, in order to prevent the increase resistance of pathogens to triclosan.

Previously study suggested effectively upregulated efflux pump genes played an important role in biocides non-susceptibility bacteria [28]. In this respect, we tested the activity of 4 different types of Tolc and their relative expressions. Under triclosan stimulation, increased efflux pump activity of the isolates were found in our study, and the expression levels of ABC transporters system encoding genes *ydcT*, *ydcU*, *ydcV*, *ydcS*, and *cysU* and RND-type tolc encoding genes *acrB*, *acrD* and *yihV*, as well as *mdfA* gene which belonging to MFS family had a significant increase compared with ATCC 25922, which were consistent with a previous study in China [29]. In contrast to previous studies, we did not observed any increase in the expression levels of other efflux pump encoding genes, maybe it can be understood that ABC transport efflux pump, RND-type tolc and MFS family activity a stronger advantage than others during making adaptive changes of the studied isolates to triclosan [17, 22]. All in all, we found that there was a strong relationship between genes overexpression and the increased tolerance of *E. coli* against triclosan, which suggested multiple efflux pumps may synergistically mediate triclosan resistance.

Similar to previous reports, we found that 5 triclosan resistance strains showed *fabI* overproduction [29]. Nevertheless, we did not observe any sense mutations within *fabI* gene in triclosan resistant strains, which was inconsistent with other reports [16, 17]. Perhaps, triclosan resistance is mediated by *fabI* and efflux pumps overexpression in our tested strains, rather than *fabI* mutation. In addition, different pulse types and STs types were observed in the studied isolates by PFGE and MLST, suggesting that there was no transmission and a clonal dissemination among these triclosan resistant strains.

However, our study also has some limitations. Although multiple and cross-resistance between triclosan and antibiotics were noticed, we did not illuminate the underlying mechanisms, which is the focus of our future research.

Conclusions

We first put forward that *E. coli* isolates can acquire triclosan resistance only 4 days under the stimulation of triclosan with subinhibitory in vitro, and triclosan resistant strains often showed multidrug or cross-resistance profiles which may be related to exposure time of triclosan. In addition, we also systematically explained that bacteria are resistant to triclosan through *fabI* and efflux pumps overexpression. In short, our research emphasize, with extensive and long-term use of triclosan, the need for improved vigilance of the presence and emergence of multidrug resistant *E. coli* bacteria in urine, moreover, the rational use of triclosan is also a necessary measure to control the spread of multiple and cross-resistance.

Abbreviations

ATCC:American Type Cultures Collection; CLSI:Clinical and Laboratory Standards Institute; CCCP:Carbonyl cyanide 3-chlorophenylhydrazone; CDC:Centers for Disease Control and Prevention; *E. coli*:*Escherichia coli*; MICs:Minimal Inhibitory Concentrations; MDR:Multidrug-resistant; MIC_{90}:The concentration required to inhibit growth by 90% isolates; MLST:Multilocus sequence typing; MATE:The multidrug and toxic compound extrusion family; MFS:The

major facilitator superfamily; LB:Luria Broth; PCR:Polymerase chain reaction; PFGE:Pulse field gel electrophoresis; RT-qPCR:Quantitative real-time polymerase chain reaction; RND:The resistance nodulation division family; SSIs:Surgical site infections; STs:Sequence types; SMR:The staphylococcal multi-resistance family; UTI:Urinary tract infection.

Declarations

Ethics approval and consent to participate

The need for ethics approval and consent is deemed unnecessary in this research according to the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Contributions

WLZ, WYX, and WLL carried out experiments. WLZ and YX analyzed the data. WLZ wrote the manuscript. CQX and XKZ performed the results analysis and YJZ directed the drawing. JMC and TLZ designed the study and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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Supplementary information

Additional file 1: Table S1. The primers used in this study. Tables S2. Relative genes expression in *E. coli* ATCC 25922 and the field triclosan resistant isolates. Tables S3. MICs of triclosan and antibiotics against *E. coli* wild-type and mutant strains.

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Figures

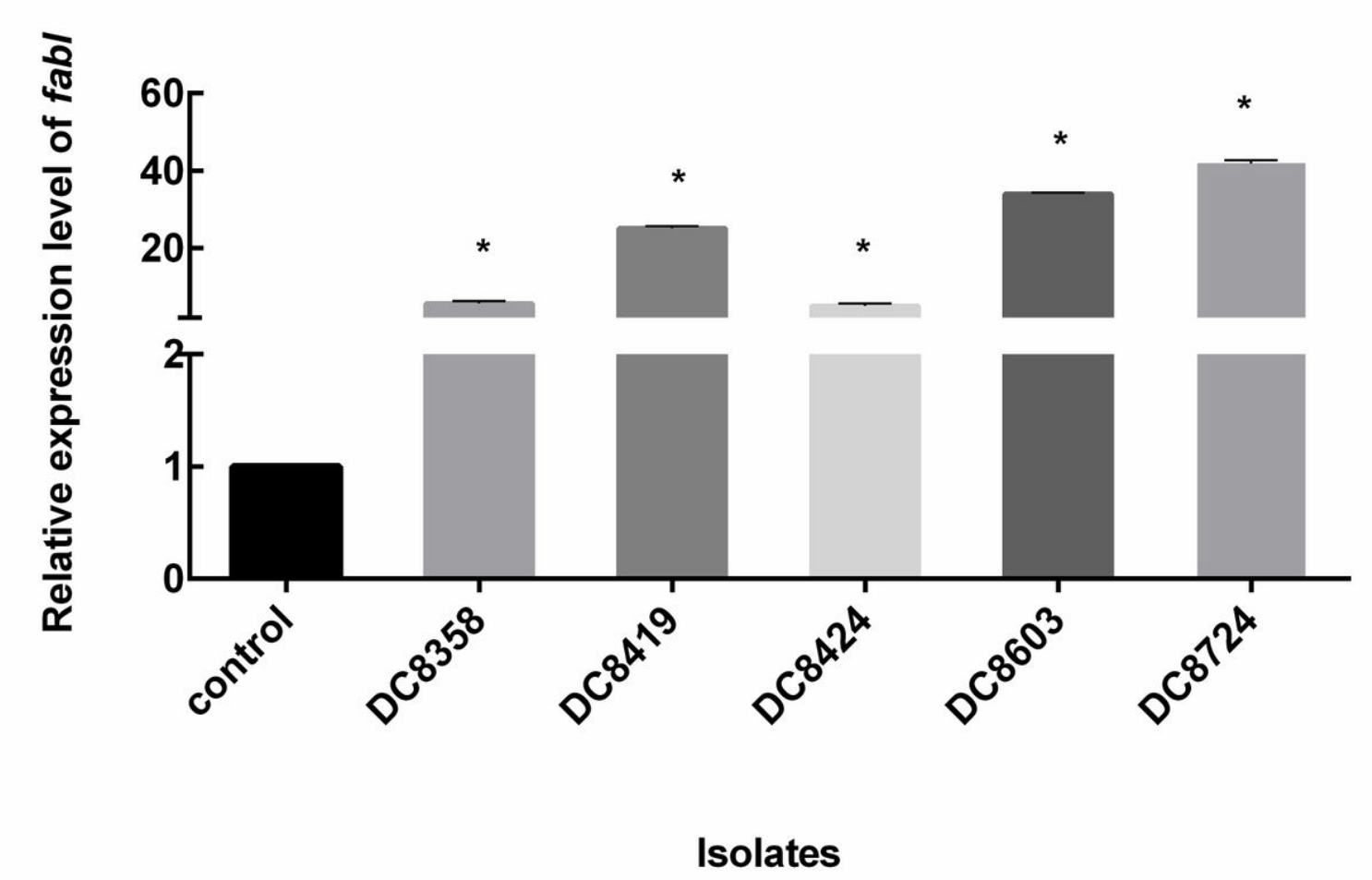

Figure 1

Relative expression level of fabI Values of three biological repeats represent the mean ± SD. * means gene overexpression, that was the relative expression levels increased by 2-fold or greater compared to the control strain E. coli ATCC 25922. Compared to ATCC 25922, the expression of fabI was increased (> 2-fold) in DC8358 (fold-changes: 5.69 ± 0.49), DC8419 (fold-changes: 25.14 ± 0.42), DC8424 (fold-changes: 5.11 ± 0.43), DC8603 (fold-changes: 34.05 ± 0.23) and DC8724 (fold-changes: 41.85 ± 0.59).

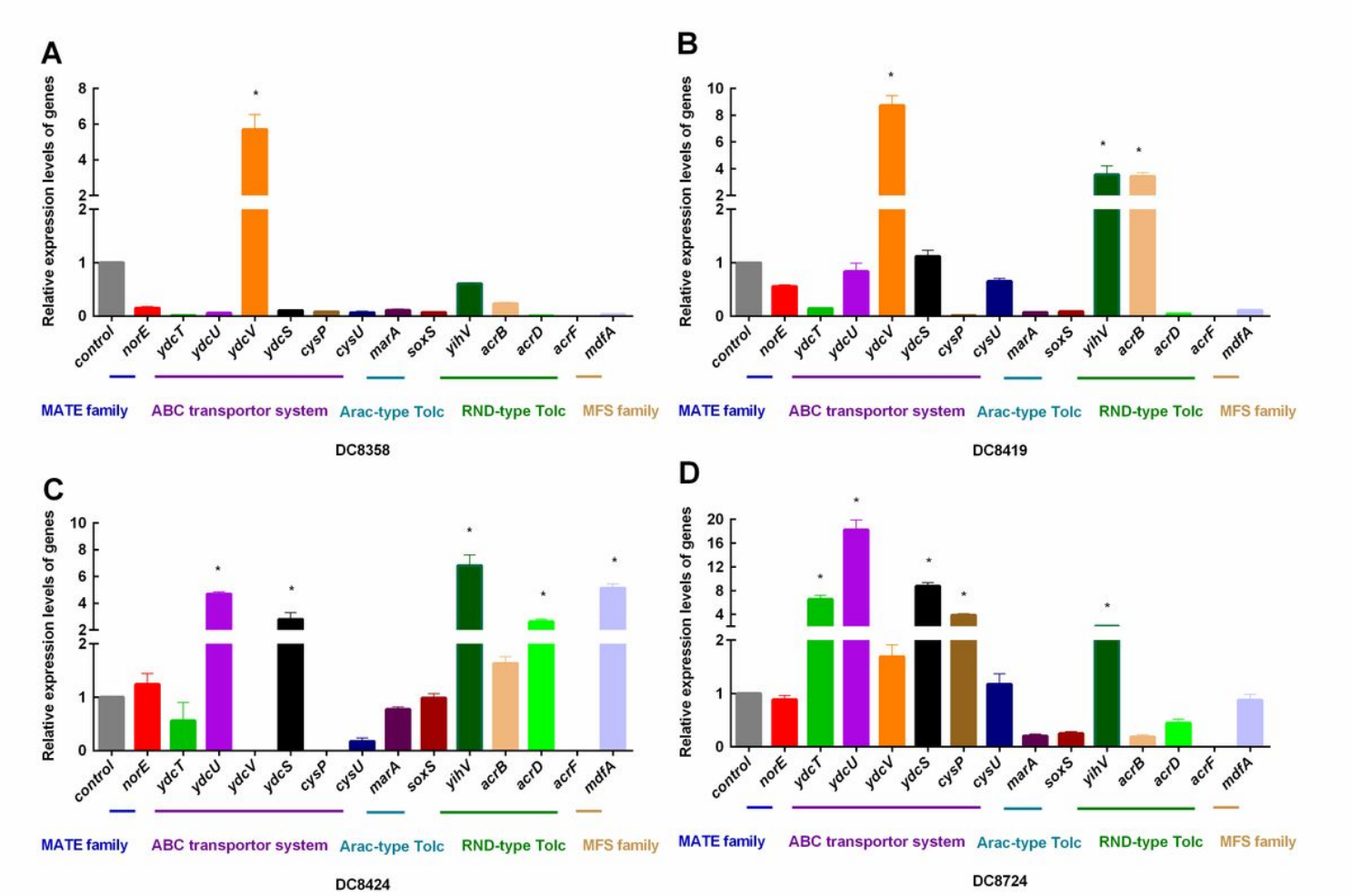

Figure 2

Relative expression levels of genes (A) The relative expression levels of efflux pump encoding genes in DC8358, the expression of ydcV (fold-changes: ydcV, 5.71 ± 0.68) was increased; (B) The relative expression levels of efflux pump encoding genes in DC8419, the expression of ydcV, yihV and acrB (fold-changes: ydcV, 8.74 ± 0.61; yihV, 3.57 ± 0.52; acrB, 3.44 ± 0.21, respectively) was increased; (C) The relative expression levels of efflux pump encoding genes in DC8424, the expression of ydcU, ydcS, yihV, acrD, and mdfA (fold-changes: ydcU, 4.71 ± 0.13; ydcS, 2.8 ± 0.42; yihV, 6.82 ± 0.65; acrD, 2.63 ± 0.14; mdfA, 5.13 ± 0.26, respectively) was increased; (D) The relative expression levels of efflux pump encoding genes in DC8724, the expression of ydcT, ydcU, ydcS, cysP, and yihV (fold-changes: ydcT, 6.56 ± 0.56; ydcU, 18.25 ± 1.36; ydcS, 8.76 ± 0.49; cysP, 3.89 ± 0.2; yihV, 2.00 ± 0.03, respectively) was increased. Values of three biological repeats represent the mean ± SD. * means gene overexpression, that was the relative expression levels increased by 2-fold or greater compared to the control strain ATCC 25922.

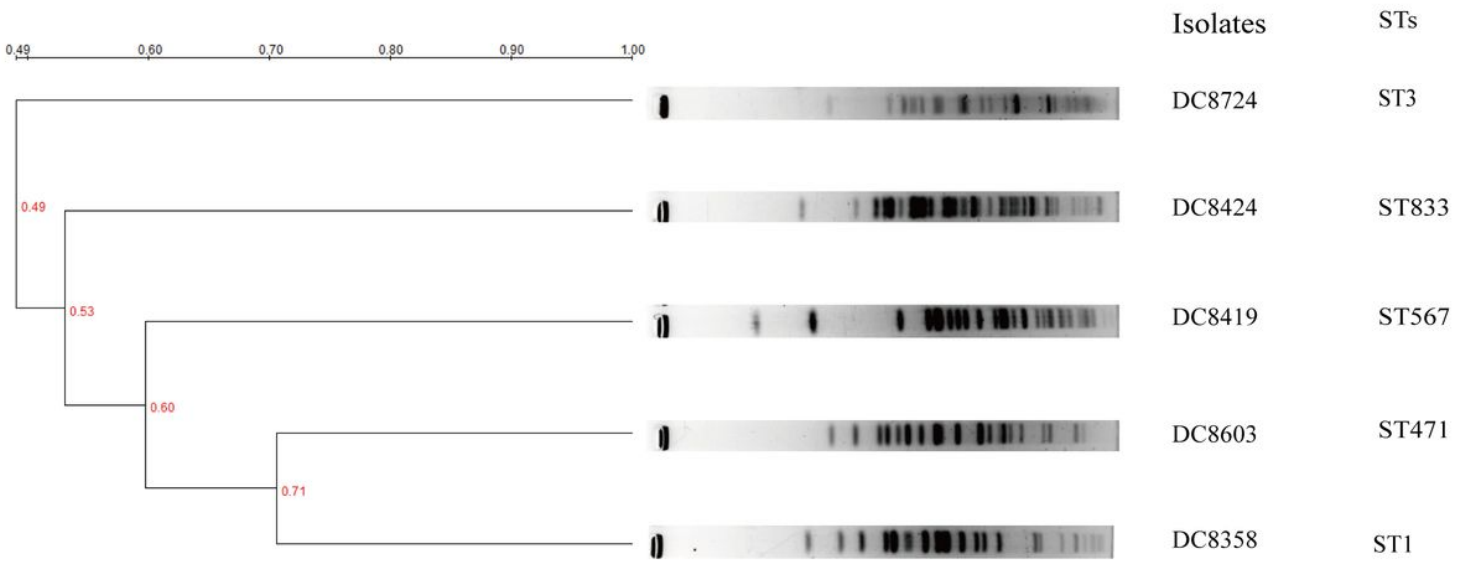

Figure 3

PFGE and MLST profile of triclosan resistant E. coli isolates Relatedness of PFGE results was analyzed using QualityOne software (Bio-Rad Laboratories, USA), and the phylogenetic tree was generated using UPGMA clustering, cut off line ≥ 85% was considered to analyze genetic relatedness. The result showed differences in PFGE patterns and STs typing.

Supplementary Files

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