

**Conjugative transfer of plasmid p\_8N\_qac(MN687830.1) carrying *qacA* gene from *Staphylococcus aureus* to *Escherichia coli* C600: potential mechanism for spreading chlorhexidine resistance**

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**ABSTRACT**

The methicillin resistant *Staphylococcus aureus* (MRSA) is recognized by its ability to acquire and transferring resistance genes through interspecies conjugative plasmids. However, transference of plasmids from Gram-positive cocci to Gram-negative bacilli is not well characterized. In this report, we describe the transfer of a conjugative plasmid carrying *qacA* from MRSA to *Escherichia coli* C600. We performed a conjugation experiment using a chlorhexidine resistant MRSA isolate (ST-105/SCCmec type III) carrying the gene *qacA* and *qacC* as the donor and a chlorhexidine susceptible *E. coli* C600 isolate as the receptor. Transconjugants were selected using MacConkey agar plates containing chlorhexidine in concentrations ranging from 0.25 to 16 g.L<sup>-1</sup>. To genotypically confirm the transfer of the resistance gene, the transconjugants were screened by Polymerase Chain Reaction (PCR) and submitted to Sanger's sequencing. MRSA isolates successfully transferred the chlorhexidine resistance gene (*qacA*) to the recipient *E. coli* strain C600. The *E. coli* transconjugant exhibited an important reduction of chlorhexidine susceptibility, with MICs increasing from ≤ 0.25 to ≥ 16 g.L<sup>-1</sup> after conjugation. The *qacA* gene was detected by PCR as well as in the Sanger's sequencing analysis of DNA from transconjugant plasmids. To the best of our knowledge, this is the first report of the plasmid p\_8N\_qac(MN687830.1) carrying *qacA* and its transfer by conjugation from a MRSA to an *E. coli*. These findings increase concerns on the emergence of resistance dissemination across the genus and emphasizes the importance of continuous antiseptic stewardship.

**KEYWORDS:** Chlorhexidine resistance. Conjugation. Conjugative plasmids. *qacA*.

**INTRODUCTION**

Conjugative plasmids are the main carriers of antibiotic resistance genes. They play a significant role in resistance dissemination and bacterial evolution by accelerating adaptation through horizontal gene transfer, thus providing an opportunity for recombination<sup>1</sup>.

An example of resistance spreading by transfer of mobile genetic elements is methicillin-resistant *Staphylococcus aureus* (MRSA), a common etiological agent of healthcare associated infections (HAI), with a high antibiotic resistance rate<sup>2,3</sup>. Its ability to acquire and transfer resistance genes and virulence determinants through interspecies conjugative plasmids poses a serious concern for public health. Enterobacteria, such as *Escherichia coli*, were previously known to acquire mobile

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genetic elements from other genus and are also capable of disseminating these elements containing antimicrobial resistance genes through horizontal transfer<sup>4</sup>. However, studies about spreading of resistance genes from Gram-positive to Gram-negative bacteria are limited. HAI among critically ill patients have been shown to increase the risk of morbidity, mortality, as well as the healthcare costs. Some of the approaches to decrease the risk of infection and to prevent resistance dissemination include pre-operative cleansing and daily bathing with chlorhexidine, an ammonium quaternary compound. Bacterial exposure to chlorhexidine results in cellular membrane rupture followed by bacterial death, promoting only minimal skin irritation<sup>5</sup>. Although this strategy is widely used in several countries, its effectiveness remains uncertain and there are concerns if it can promote chlorhexidine resistance<sup>6</sup>. Resistance to ammonium quaternary compounds is typically mediated by three genes *qacA*, *qacB* and *qacC/smr*. The gene *qacA* encodes an efflux pump that confers export-mediated resistance and its presence was associated with a phenotype of reduced susceptibility towards several biocide compounds<sup>7</sup>. The increased efflux of antibiotics by the outer membrane proteins has gained importance due to their multi-specificity that allows to expel a large number of unrelated substrates<sup>8</sup>.

The emergence of multi-drug resistance by transfer of mobile genetic elements from MRSA to enteric pathogens was proposed as a potential concern by Russell<sup>9</sup>, and now, more than two decades later, we describe the transfer of a conjugative plasmid between a MRSA and an *E. coli* C600 isolate, the former carrying the *qacA* gene. This study aimed to report and investigate the role of transferable plasmids between Gram-positive and Gram-negative bacteria.

## MATERIALS AND METHODS

The present study includes an isolate from a previous study conducted by Bes *et al.*<sup>10</sup>, who identified chlorhexidine resistance among clinical MRSA isolates. Here, a conjugation assay was performed using as the donor an isolate, labeled as 8N, that had been previously submitted to Whole Genome Sequencing (WGS) by Illumina to characterize its resistance and virulence profile. The 8N isolate was identified as a chlorhexidine resistant MRSA, assigned as ST-105, *SCCmec* III, carrying the genes *qacA* and *qacC*. In addition to chlorhexidine resistance, the isolate carried *ermA*, *ermC*, *mphC*, *msrA*, *norA* and *mecA* conferring resistance to erythromycin, clindamycin, ciprofloxacin and oxacillin<sup>10</sup>. As recipient, we used an *Escherichia coli* C600 isolate from the American Type Culture Collection (ATCC 23724). *E. coli* strain C600,

primarily described in 1954, is derived from a progenitor strain K-12, first isolated in 1922. It is a laboratory strain that lacks plasmids or restriction enzyme modification systems<sup>11</sup>.

A broth mating experiment was performed among a chlorhexidine resistant MRSA isolate carrying the *qacA* gene and a chlorhexidine susceptible *E. coli* C600 isolate, applying an adapted protocol from Ferrières *et al.*<sup>12</sup>. Donor and recipient cultures had grown overnight at 37 °C in MacConkey and blood agar medium for the *E. coli* and for the MRSA isolate, respectively. Up to three colonies of both isolates were transferred individually from the previous culture plate to a fresh 2 mL of Tryptic Soy Broth (TSB) and were incubated at 37 °C for approximately 4 h until cultures reached the OD 600 of 1.2 (corresponding to approximately 10<sup>9</sup> CFU/mL). As controls, recipient and donor isolates were cultured individually and handled similarly to the conjugation assay.

For broth culture matings, the cultures were mixed at a 1:10 ratio of donor (100 uL) and recipient (1,000 uL) cells in fresh TSB (4ml) and incubated at 37 °C without agitation for 18 h, 24 h and 48 h. For the controls, the TBS fresh solution was added to 1,000 uL of the donor culture and 100 uL of the recipient culture, in addition to 100 uL and 1,000 uL of phosphate-buffered saline (PBS), for the donor and recipient cultures, respectively. During the incubation endpoints, 100 uL of the donor, recipient and control cultures were plated on MacConkey agar plates with chlorhexidine in different concentrations, ranged from 0.25 to 16 g L<sup>-1</sup>, using as reference the agar dilution method<sup>13</sup> and incubated at 37 °C for 24 h.

To verify the plasmid transfer, the transconjugants were submitted to genotypic and phenotypic tests. Phenotypically, the expression of the *qacA* gene was confirmed by comparing susceptibility profiles of controls and transconjugants. The recipient strain *E. coli* C600 was susceptible to chlorhexidine prior to conjugation (MIC < 0.25) and the donor strain MRSA was resistant to chlorhexidine (MIC: 8).

Genotypically, to verify if the plasmid was successfully transferred to the recipient, the transconjugants were harvested from the MacConkey agar plates and the plasmid DNA was extracted using the QIAGEN extraction kit, QIAprep Spin Miniprep (QIAGEN Inc., Hilden, Germany). Then, the extracted plasmid DNA was used for the screening of *qacA* genes by Polymerase Chain Reaction (PCR), using the forward primer 5'-CTATGGCAATAGGAGATATGGTGT and the reverse primer 5'-CCACTACAGATTCTTCAGCTACATG, as previously described<sup>14</sup>. To confirm the outcome, the agarose gel bands were submitted to DNA extraction and sequenced

by the Sanger's method using MegaBACE 1000 (ABI 3730 DNA Analyser: Applied Biosystems, Thermo Fisher Scientific, Waltham, USA).

## RESULTS

The conjugation assays demonstrated that the chlorhexidine resistance gene from the MRSA (ST-105 SCCmec type III) isolate could be transferred to a recipient *E. coli* strain C600. The endpoint of 18 h was selected as it corresponded to the best conjugation in comparison with longer time endpoints. All *E. coli* transconjugants exhibited significant reductions in the chlorhexidine susceptibility, which raised from a MIC of  $\leq 0.25$  to  $\geq 16$  g. L<sup>-1</sup> after conjugation. The experiments, performed in triplicate and in two different trials, presented consistent results, indicating that chlorhexidine resistance genes are capable of conjugal transfer even among different species. The successful plasmid transfer to the recipient was verified by PCR and subsequently by Sanger's sequencing analysis, which confirmed the presence of *qacA* gene in the transconjugant isolates.

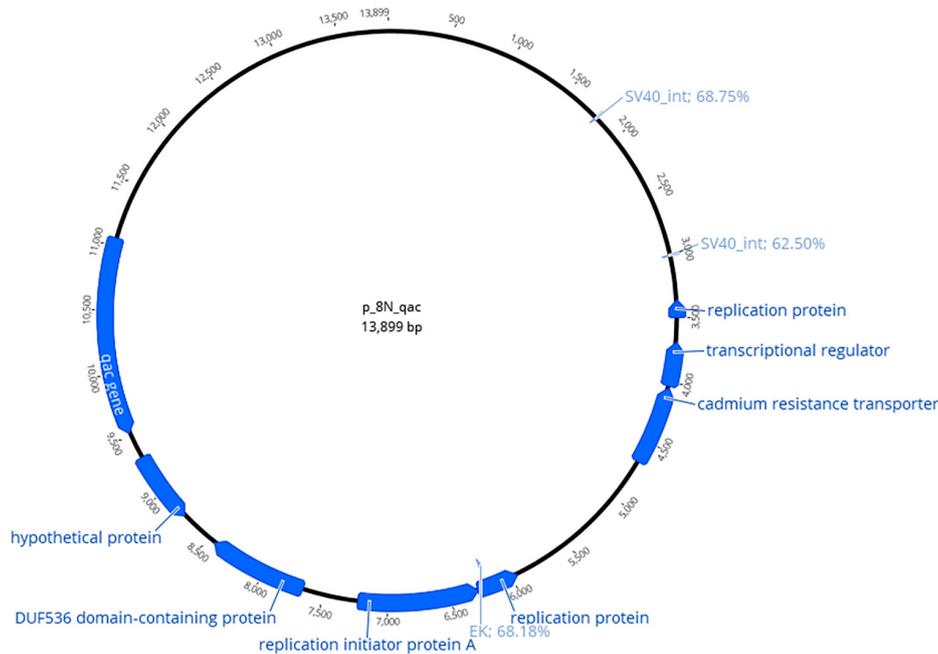
Using Illumina assemblies, it was possible to determine the complete sequence of the plasmid from the transconjugant isolate (GenBank accession number for this nucleotide sequence is MN687830, [Supplementary Material](#)). The genome analysis has shown a plasmid sequence with 13,899 base pairs and two origins of replication (rep20 and rep2), named p\_8N\_qac ([Figure 1](#)), which showed high nucleotide

identity (97.4%) with pSAZ\_10A(SDOC01000025), a *qacA* bearing a plasmid from an MRSA ST-239 strain isolated from a wound drainage material of a patient in Malaysia<sup>15</sup>.

## DISCUSSION

In this study, we described a conjugative mobilization of the plasmid p\_8N\_qac (MN687830.1) carrying the *qacA* gene from a chlorhexidine resistant MRSA isolate to a chlorhexidine susceptible *E. coli* C600 isolate. To our knowledge, this is the first report highlighting the *qacA* gene conjugation from a MRSA isolate to a Gram-negative bacillus and the first description of the plasmid p\_8N\_qac carrying *qacA*. Previously, *qacA* has only been found in larger non-conjugative plasmids, such as the ones from the pSKI family, which lack the transfer gene required for conjugation<sup>16</sup>.

Interspecies conjugation was shown by Udo and Grubb<sup>16</sup>, who reported a conjugation experiment with plasmids pWBG636 (GmR) and pWBG642 (EmR) derived from the conjugative plasmid family pWBG637. These plasmids were transferred from *S. aureus* to *S. epidermidis* and *E. faecalis* strains but were unsuccessful from *S. aureus* to *E. coli*. Then, Charpentier *et al.*<sup>17</sup> in 1999 reported the transfer of a conjugative plasmid pIP823 (plasmid family: pUB110/pC194) which contained the *dfpD* gene (conferring a high-level of trimethoprim resistance) from *Listeria monocytogenes* to Gram-negative and Gram-positive bacilli, specifically *E. faecalis* and *E. coli*<sup>17</sup>.



**Figure 1** - Simplified pSAZ\_10A(SDOC01000025) plasmid map. Regions amplified during the Sanger's sequencing screen are indicated by blue boxes along the plasmid backbone, as well as our gene of interest: *qacA*.

In our report, conjugation assays showed that the determinant of chlorhexidine resistance in MRSA isolates can be transferred to the recipient *E. coli* (C600 strain). LaBreck *et al.*<sup>18</sup> showed the ability of *qacA* to be mobilized across *Staphylococcus spp.* strains, displaying a conjugative transfer of *qacA*-positive pC02 plasmid (pWBG749-family of conjugative plasmids) to several *S. aureus* and *S. epidermidis* isolates among military members from Bethesda, USA, confirmed by WGS. These previous reports make our findings even more thought-provoking, considering that our receptor isolate is a Gram-negative bacillus. Likewise, chlorhexidine is commonly used in pre-operative cleansing and daily bathing as a decolonization strategy to decrease the infection rates by MRSA and other multi-drug resistant Gram-negative bacteria<sup>6</sup>, which are responsible for most of the life threatening infections among severely ill patients in different settings<sup>19,20</sup>.

The hypothesis of horizontal transfer of resistance genes was proposed to explain the spreading of chlorhexidine resistance among enterobacteria, being the *qacA* gene transfer through a plasmid-facilitated efflux pump one possibility, which could confer export-mediated resistance across the genus.

## CONCLUSION

Herein we are reporting the first evidence of conjugative transfer of *qacA* from a Gram-positive coccus to a Gram-negative bacillus, in addition to indicating that the conjugative transfer is sufficient to decrease the susceptibility to chlorhexidine among recipient isolates. Although the present experiment does not allow us to confirm that chlorhexidine resistance is caused by plasmids dissemination, we are proposing this hypothesis for futures studies and highlighting the importance of continuous antiseptic stewardship.

## CONFLICT OF INTERESTS

None to declare.

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