Identification of the Multiresistance Gene poxtA in Oxazolidinone-Susceptible Staphylococcus haemolyticus and Staphylococcus saprophyticus of Pig and Feed Origins

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Abstract: Previous studies on the prevalence and transmission mechanism of oxazolidinone resistance gene poxtA in CoNS are lacking, which this study addresses. By screening 763 CoNS isolates from different sources of several livestock farms in Guangdong, China, 2018–2020, we identified that the poxtA was present in seven CoNS isolates of pig and feed origins. Species identification and multilocus sequence typing (MLST) confirmed that seven poxtA-positive CoNS isolates were composed of five ST64-Staphylococcus haemolyticus and two Staphylococcus saprophyticus isolates. All poxtA-positive Staphylococcus haemolyticus isolates shared similar pulsed-field gel electrophoresis (PFGE) patterns. Transformation assays demonstrated all poxtA-positive isolates were able to transfer poxtA gene to Staphylococcus aureus RN4220. S1-PFGE and whole-genome sequencing (WGS) revealed the presence of poxtA-carrying plasmids in size around 54.7 kb. The plasmid pY80 was 55,758 bp in size and harbored the heavy metal resistance gene czcD and antimicrobial resistance genes, poxtA, aadD, fexB and tet(L). The regions (IS1216E-poxtA-IS1216E) in plasmid pY80 were identified in Staphylococcus spp. and Enterococcus spp. with different genetic and source backgrounds. In conclusion, this was the first report about the poxtA gene in Staphylococcus haemolyticus and Staphylococcus saprophyticus, and IS1216 may play an important role in the dissemination of poxtA among different Gram-positive bacteria.

Keywords: poxtA; CoNS; transformation; plasmids; antimicrobial; heavy metal; IS1216

1. Introduction

Coagulase negative staphylococci (CoNS) are one of the most common opportunistic pathogens found on human skin and mucous membranes as a component of normal flora [1,2]. Besides their role in keeping homeostasis, CoNS have been involved in a series of infectious processes, ranging from nosocomial infections to livestock bacterial sepsis and mastitis [3,4]. In addition to their virulence, the emergence of antibiotic resistance in CoNS and then horizontal dissemination among staphylococci should be alarming. The increasing drug resistance of CoNS significantly limited the treatment options [5,6]. Among CoNS, Staphylococcus haemolyticus is the second most frequently isolated from human blood culture and Staphylococcus saprophyticus is one of the most common pathogens responsible for community urinary tract infections [5,7].
Oxazolidinones such as linezolid and tedizolid are antibacterially active against Gram-positive pathogens including methicillin-resistant Staphylococcus aureus (MRSA), methicillin-resistant CoNS and vancomycin-resistant enterococci (VRE) [8]. However, the discovery of transferable oxazolidinone resistance genes such as cfr, cfr(B), cfr(C), optrA and poxtA as well as the mutations in 23S rRNA and ribosomal proteins L3 and L4 challenged the clinical use of oxazolidinones [9]. Worriedly, linezolid-resistant staphylococci have been detected worldwide [10]. The plasmid-mediated oxazolidinone resistance genes including cfr and optrA spread among a number of bacterial species of different origins around the world shortly after they were reported [11]. The fact that cfr and optrA genes can be selected by phenicols and other ribosomal-targeted drugs that are widely used in livestock and veterinary hospitals is closely associated with global spread of the resistance genes [12–14]. The recently described plasmid-mediated oxazolidinone resistance gene poxtA could decrease susceptibility to phenicols and tetracyclines, so the poxtA gene posed a threat to disseminate in bacteria from animal setting [15,16]. The poxtA gene has been identified in MRSA and Enterococcus strains of human and animal origins [15–18]. Livestock is widely recognized as a reservoir of antimicrobial resistance genes [19].

In this study, we described for the first time the identification and characterization of poxtA gene in S. haemolyticus and S. saprophyticus isolates from pig and chicken farms in Guangdong province, China.

2. Results
2.1. Identification of poxtA Gene in CoNS Isolates
The poxtA gene was detected in seven CoNS isolates in 2018 including five S. haemolyticus isolates (GDY8P33P, GDY8P50P, GDY8P58P, GDY8P60P and GDY8P80P all of pig origin) and two S. saprophyticus isolates (GDY8P136P of pig origin, GDH8C97P of feed origin) (Table 1).

Table 1. Background information on the 7 CoNS isolates carrying the poxtA gene.

| Isolate       | Sampling Time | Origin (Farm Type) | Species       | MLST |
|---------------|---------------|--------------------|---------------|------|
| GDH8C97P      | June 2018     | Feed sample (chicken farm A) | S. saprophyticus | –    |
| GDY8P33P      | December 2018 | swine nasal swab (pig farm D) | S. haemolyticus | ST64 |
| GDY8P50P      | December 2018 | swine nasal swab (pig farm D) | S. haemolyticus | ST64 |
| GDY8058P      | December 2018 | swine nasal swab (pig farm D) | S. haemolyticus | ST64 |
| GDY8P60P      | December 2018 | swine nasal swab (pig farm D) | S. haemolyticus | ST64 |
| GDY8P80P      | December 2018 | swine nasal swab (pig farm D) | S. haemolyticus | ST64 |
| GDY8P136P     | December 2018 | swine nasal swab (pig farm D) | S. saprophyticus | –    |

MLST: “–” indicates that S. saprophyticus cannot be typed by MLST.

2.2. Distribution of ARGs in poxtA-Positive CoNS Isolates and the Electrotransformants
In total, 16 additional ARGs were detected among the poxtA-positive CoNS isolates (Figure 1). ARGs were widespread in the poxtA-positive isolates of both feed and pig origins. Except for the widely distributed resistance genes aadD, ant(6)-Ia, blaZ, mecA, Isa(E), lnu(B), erm(C), fecB, tet(L) and dfrG in the poxtA-positive CoNS isolates, the distribution of individual ARG varied among the poxtA-positive CoNS isolates. For example, the cfr, tet(M) and aac(6')-aph(2") genes were also identified in the poxtA-positive S. haemolyticus isolates (Figure 1). All poxtA-positive CoNS isolates were able to transfer the fecB, poxtA and tet(L) genes to S. aureus strain RN4220 (Table 2). In addition, the five poxtA-positive S. haemolyticus isolates were able to transfer the aadD gene to S. aureus strain RN4220 (Table 2).

2.3. Antimicrobial Susceptibility
Antimicrobial susceptibility testing showed that resistance rates of seven poxtA-positive CoNS isolates to penicillin, cefoxitin, doxycycline, tetracycline, florfenicol, erythromycin and ciprofloxacin reached 100% (Table 2). Six (85.7%) poxtA-positive CoNS isolates demonstrated resistance to enrofloxacin. All poxtA-positive CoNS isolates re-
mained susceptible to linezolid, tedizolid, tigecycline, amikacin, gentamicin, rifampicin and vancomycin. All poxtA-positive CoNS isolates were able to transfer the florfenicol resistance to S. aureus strain RN4220. Two electrotransformants are erythromycin resistant (Table 2). In addition, the electrotransformants carrying aadD, poxtA and tet(L) genes exhibited lower MICs of neomycin, kanamycin, linezolid, doxycycline and tetracycline compared with the donors (Table 2).

2.4. Phylogenetic Relatedness of poxtA-Positive CoNS Isolates

All poxtA-positive S. haemolyticus isolates derived from swine nasal swabs in pig farm D represented ST64 by MLST analysis and were closely related by phylogenetic analysis (Table 1 and Figure 1). In addition, 121 SNPs were identified in all poxtA-positive S. haemolyticus isolates (Figure 1). The S. saprophyticus isolates GDH8C97P recovered from feed in chicken farm A and GDY8P136P recovered from swine nasal swab in pig farm D shared 1957 SNPs difference (Figure 1).

2.5. Plasmids Analysis

S1-PFGE and WGS analysis confirmed that the poxtA gene in the seven CoNS isolates and corresponding electrotransformants was located on plasmids ranging in size around 54.7 kb (Figure 2 and Figure S1). Plasmid pY80 carrying the poxtA gene was 55,758 bp in size and exhibited <38% coverage with other plasmids in NCBI database, with an average GC content of 34.0%. In total, 51 ORFs coding for proteins of >50 amino acids were identified (Figure 2). Except for the 14 ORFs encoding hypothetical proteins with no defined function, the products of the remaining 37 ORFs exhibited identities ranging from 76.3% to 100% to proteins with known functions, including antimicrobial resistance, heavy metal resistance, conjugative transfer or transposition, plasmid replication and other function (Figure 2).

Figure 1. Genomic analysis of five Staphylococcus haemolyticus isolates carrying poxtA and cfr and two Staphylococcus saprophyticus isolates carrying poxtA of various origins in Guangdong, China. Phylogenetic tree was constructed using CSI Phylogeny 1.4. Sources of the isolates are indicated by different colors for geographic regions (squares), farms (stars), hosts (circles) and sample origins (squares). Antimicrobial resistance genes are indicated by the following method: purple, positive; white, negative.
Table 2. Characterization of poxtA-positive strains, their electrotransformants and the recipient strain.

| Bacterial Isolate | AMO | PEN | FOX | GEN | AMI | NEO | KAN | DOX | TET | TIG | FFC | ERY | RIF | VAN | CIP | ENR | LZD | TZD | SXT |
|-------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| S. aureus RN4220  | 0.125 | 0.125 | 2 | 0.25 | 0.25 | 0.25 | 0.125 | 0.5 | 0.06 | 2 | 0.25 | 0.008 | 1 | 0.5 | 0.25 | 0.5 | 0.06 | 0.25 |
| GDH8C97P          | 8 | 16 | 8 | 0.125 | 0.25 | 1 | 0.25 | >64 | >64 | 0.25 | >64 | >256 | 0.015 | 1 | 4 | 16 | 0.5 | 0.06 | 0.5 |
| RN4220/pH97      | 0.06 | 0.125 | 2 | 0.125 | 0.25 | 0.25 | 0.25 | 0.25 | 1 | 2 | 0.06 | 32 | >256 | 0.008 | 1 | 0.25 | 0.125 | 0.5 | 0.06 | 0.25 |
| GDY8F33P         | 2 | 4 | 16 | 8 | 2 | 4 | 8 | >64 | >64 | 0.25 | >64 | >256 | 0.004 | 1 | >64 | 32 | 2 | 0.06 | 2 |
| RN4220/pY33      | 0.06 | 0.125 | 2 | 0.125 | 0.25 | 1 | 0.25 | 1 | 2 | 0.125 | 32 | 0.125 | 0.008 | 1 | 0.5 | 0.125 | 0.5 | 0.06 | 0.25 |
| GDY8F50P         | 4 | 4 | 32 | 8 | 2 | 4 | 8 | >64 | >64 | 0.25 | >64 | >256 | 0.004 | 1 | >64 | >64 | 2 | 0.25 | 8 |
| RN4220/pY50      | 0.06 | 0.125 | 2 | 0.125 | 0.25 | 1 | 0.25 | 2 | 2 | 0.125 | 32 | 0.125 | 0.008 | 1 | 0.5 | 0.125 | 0.5 | 0.06 | 0.25 |
| GDY8F55P         | 4 | 4 | 32 | 8 | 2 | 4 | 8 | >64 | >64 | 0.25 | >64 | >256 | 0.004 | 1 | >64 | >64 | 4 | 0.5 | 16 |
| RN4220/pY58      | 0.06 | 0.125 | 2 | 0.125 | 2 | 1 | 0.25 | 2 | 1 | 0.125 | 32 | 0.125 | 0.008 | 1 | 0.5 | 0.125 | 0.5 | 0.06 | 0.25 |
| GGY8F60P         | 2 | 1 | 32 | 8 | 2 | 4 | 8 | 64 | 64 | 0.25 | >64 | >256 | 0.008 | 1 | >64 | >64 | 2 | 0.5 | 8 |
| RN4220/pY60      | 0.06 | 0.125 | 2 | 0.125 | 2 | 1 | 0.25 | 1 | 1 | 0.25 | 32 | >256 | 0.002 | 1 | 0.5 | 0.125 | 0.5 | 0.06 | 0.25 |
| GGY8F80P         | 2 | 4 | 16 | 8 | 2 | 4 | 8 | 64 | >64 | 0.25 | >64 | >256 | 0.004 | 1 | >64 | >64 | 4 | 0.25 | 16 |
| RN4220/pY80      | 0.06 | 0.125 | 2 | 0.125 | 2 | 1 | 0.25 | 1 | 2 | 0.25 | 32 | 0.125 | 0.008 | 1 | 0.5 | 0.125 | 0.5 | 0.06 | 0.25 |
| GGY8F136P        | 16 | 16 | 16 | 8 | 0.5 | 4 | 8 | 32 | 64 | 0.25 | 64 | >256 | 0.03 | 2 | 4 | 2 | 2 | 0.25 | 1 |
| RN4220/pY136     | 0.06 | 0.125 | 1 | 0.125 | 0.25 | 0.25 | 0.06 | 0.2 | 0.03 | 16 | 0.125 | 0.002 | 1 | 0.25 | 0.125 | 0.5 | 0.06 | 0.25 |

AMO, amoxicillin; PEN, penicillin; FOX, cefoxitin; GEN, gentamicin; AMI, NEO, neomycin; KAN, kanamycin; amikacin; DOX, doxycycline; TET, tetracycline; TIG, tigecycline; FFC, florfenicol; ERY, erythromycin; RIF, rifampicin; VAN, vancomycin; CIP, ciprofloxacin; ENR, enrofloxacin; LZD, linezolid; TZD, tedizolid; SXT, trimethoprim-sulfamethoxazole. Resistance genes: “–” indicates that no resistance gene was shown in the area. MICs (mg/L) shaded grey represent strains that were resistant to the corresponding antimicrobial agents; despite the lack of clinical breakpoints applicable to staphylococci, the MICs of neomycin and kanamycin were detected in poxtA-positive strains, their electrotransformants and the recipient strain S. aureus RN4220.
2.6. Genetic Environment of \textit{poxtA} Gene

The \textit{poxtA}-carrying segments (IS1216-\textit{poxtA}-IS1216-\textit{fexB}-IS431mec-tet(L)-aadD-IS431mec) of 17287 bp in plasmid pY80 of pig origin were selected to conduct comparative analysis with other \textit{poxtA}-carrying segments. The IS1216-\textit{poxtA}-IS1216 segment of 4130 bp showed >98% identity to corresponding sequences in two \textit{Enterococcus hirae} plasmids (pHDC14-2.27K and pfas4-1 both of pig origins), two \textit{Enterococcus faecalis} plasmids (pM18/0011 of human origin and pC10 of pig origin), 10 \textit{Enterococcus faecium} plasmids (pSDGJQ5 of chicken origin, pM160954 of human origin, pE1077-23 of pig origin, pSCBC1 of pig origin, pSDGJP3 of pig origin, pYN2-1 of pig origin, pHN11 of pig origin, pGZ8 of pig origin, pSC3-1 of chicken origin and pC25-1 of pig origin) and the genome of \textit{S. aureus} AOUC-0915 of human origin (Figure 3). In addition, the IS1216-\textit{poxtA} segment of 2363 bp showed >98% identity to corresponding sequences in the genomes of \textit{Enterococcus faecium} pDGB of human origin) (Figure 3). The \textit{poxtA}-carrying fragments that often harbor additional resistance genes such as \textit{fexB}, tet(L) and tet(M) were identified in different bacterial species (Figure 3).
Figure 3. Comparison of the genetic contexts of poxtA in plasmid pY80 investigated in this study with corresponding sequences in other plasmids and genomic DNA. Arrows indicate the positions and orientations of the genes. Antimicrobial resistance genes are shown in red. Mobile element regions are underlined in yellow. Insertion sequences are indicated as boxes, with the arrow inside the box showing the transposase gene. Genes with unknown functions and other functions are shown in light blue. Regions of >98% nucleotide sequence identity are shaded grey. ∆ indicates an incomplete gene.

3. Discussion

CoNS are recognized as significant opportunistic pathogens that cause infections in humans and animals [4,5], and CoNS carrying important antimicrobial resistance genes such as oxazolidinone resistance genes could pose a huge burden on the healthcare system and breeding industry [10]. The transferable oxazolidinone resistance gene poxtA in different enterococci was the most recently reported [17]. Attention should be paid to the fact that the poxtA gene was originally detected in a linezolid-resistant MRSA strain [16]. Therefore, there was a risk that the poxtA could spread to other bacterial strains. The observation that the poxtA gene was mainly detected in S. haemolyticus and S. saprophyticus isolates might suggest an S. haemolyticus and S. saprophyticus reservoir. In this study, the results indicated that poxtA-positive ST64-S. haemolyticus isolates from swine nasal swabs in a pig farm shared low SNPs difference and were closely related, and that poxtA-positive S. saprophyticus isolates from a pig farm and a chicken farm shared a high SNPs difference. Therefore, ST64-S. haemolyticus isolates carrying poxtA can spread among pigs in the pig farm, and the potential spread of S. saprophyticus isolates carrying poxtA between the pig farm and chicken farm should arouse people’s attention.

It was reported that IS1216 played a major role in the processes of aiding the dissemination and persistence of poxtA among enterococci [20]. The presence of these homologous gene regions (IS1216-poxtA-IS1216 or IS1216-poxtA) in Staphylococcus spp., Enterococcus spp. and Pediococcus spp. confirmed that IS1216 was closely related to the spread of poxtA.
among these Gram-positive bacteria with different genetic and source backgrounds. This was a further reminder that the \textit{poxtA} gene might have spread widely in these bacteria. It has been found from reported studies that most of these homologous gene regions are located on transferable plasmids which often harbor additional resistance genes such as the tetracycline resistance genes \textit{tet}(M) and \textit{tet}(L), and the phenicol exporter gene \textit{fexB} [15–18]. In this study, plasmid \textit{pY80} carried heavy metal resistance gene \textit{czcD} and aminoglycoside-modifying enzyme gene \textit{aadD} in addition to \textit{tet}(L), \textit{fexB} and \textit{poxtA} genes. The co-occurrence of \textit{poxtA} with other antimicrobial and heavy metal resistance genes on the transferable plasmids may lead to the co-selection of \textit{poxtA}, contributing to its persistence and accelerating its dissemination [15]. The \textit{poxtA} gene was identified in the new plasmid. Once the \textit{poxtA} gene is inserted into a plasmid with strong transmission ability, it will bring great difficulties to control the further transmission of the \textit{poxtA} gene. In China, florfenicol has been widely used in food-producing animals [21]. It was reported that the emergence of oxazolidinone resistance genes such as \textit{poxtA} is closely related to the use of florfenicol in breeding farms [22]. Antimicrobial susceptibility testing showed that all donors and electrotransformants were resistant to florfenicol, indicating \textit{poxtA}-positive plasmids could be directly selected by florfenicol. The phenomenon that the strains and electrotransformants investigated in this study were phenotypically oxazolidinone-susceptible despite the fact that they carry up to two oxazolidinone resistance genes (\textit{cfr} and \textit{poxtA}) is very interesting. This may be due to the possibility that \textit{cfr} was not transcribed [23] and \textit{poxtA} played a relatively low role on oxazolidinone susceptibility [16]. The fact that electrotransformants with \textit{aadD} and \textit{tet}(L) genes did not show resistance to kanamycin, neomycin, doxycycline and tetracycline might be related to the silencing of these genes. It is easy for people to ignore the resistance genes without corresponding drug-resistant phenotypes, resulting in the widespread spread of them [24]. That antimicrobial agents used in livestock could exert selective pressures on bacteria [25] and all the \textit{poxtA}-positive CoNS isolates exhibited multidrug resistance and carried additional resistance genes should account for the spread of \textit{poxtA} gene in the CoNS isolates [15].

4. Materials and Methods

4.1. Bacterial Isolations and Detection of \textit{poxtA} Gene

A total of 778 CoNS isolates were collected from 1 chicken farm, 15 pig farms and 18 duck farms in Guangdong, China, between 2018–2020. Isolates were recovered from 34.6% (9/26) of human nasal swabs, 58.9% (353/599) of swine nasal swabs, 39.8% (33/83) of feed samples, 47.5% (94/198) of pond water samples, 34.3% (35/102) of soil samples, 65.4% (51/78) of airborne dust samples and animal 52.3% (203/388) of viscera samples. All isolates were screened for the presence of \textit{poxtA} by PCR using previously described primers [15]. Species identification was performed using MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany) and further confirmed by 16S rDNA sequence analysis.

4.2. Molecular Epidemiology Analysis and Transformation Assays

Multilocus sequence typing (MLST) was conducted for identification of clonal correlation of the \textit{poxtA}-positive \textit{S. haemolyticus} (\url{http://www.shaemolyticus.mlst.net} Accessed on: 29 January 2021) [26]. Plasmid DNA from all \textit{poxtA}-positive CoNS isolates was extracted using a Qiagen Prep Plasmid Midi Kit (Qiagen, Hilden, Germany) and transferred into a recipient \textit{S. aureus} strain RN4220 by electroporation using Gene Pulser apparatus (Bio-Rad, Hercules, CA, United States) [27]. Electrotransformants were selected on brain heart infusion (BHI) agar containing 10 µg/mL of florfenicol. Electrotransformants were further confirmed for the presence of \textit{poxtA} gene by PCR analysis. The successful electrotransformants were further screened for the presence of \textit{aadD}, \textit{fexB}, \textit{tet}(L) and \textit{tet}(M) genes by PCR.
4.3. Antimicrobial Susceptibility Testing

All poxtA-positive CoNS isolates and corresponding electrotransformants were investigated for their MICs of florfenicol, linezolid, amoxicillin, penicillin, cefoxitin, doxycycline, tetracycline, gentamicin, amikacin, neomycin, kanamycin, erythromycin, ciprofloxacin, enrofloxacin, rifampicin, vancomycin and trimethoprim-sulfamethoxazole by broth microdilution following the recommendations given in CLSI documents VET01-S2 and M100-S30 [28,29]. *Staphylococcus aureus* ATCC 29213 was used as the quality control strain.

4.4. S1-PFGE and WGS Analysis

Genomic DNA of all poxtA-positive CoNS isolates and corresponding electrotransformants were digested with S1 endonuclease and separated by PFGE as previously described [30]. Whole-cell DNA of all poxtA-positive CoNS isolates were prepared using the HiPure Bacterial DNA Kit (Magen, Guangzhou, China), following the manufacturer’s instructions, and then preceded by library construction on Novaseq 6,000 sequencing platform, which produced 150 bp paired-end reads (Novogene Company, Beijing, China). Novaseq sequences were assembled using CLC Genomics Workbench 10 (CLC Bio, Aarhus, Denmark). The GDY8P80P isolate carrying poxtA and cfr genes was further used for whole-genome sequencing on PacBio RS II sequencing platform (Biochip Company, Tianjin, China). Pacbio sequences were assembled using hierarchical genome-assembly process [31]. The assembled Pacbio sequences were corrected through Burrows-Wheeler Aligner’s Smith-Waterman Alignment (BWA-SW) software to ensure their integrity according to Novaseq sequences [32]. The plasmids carrying poxtA were annotated using the Rapid Annotation of microbial genomes using Subsystems Technology annotation server (http://rast.nmpdr.org/ Accessed on: 29 January 2021) [33]. Acquired resistance genes (ARGs) were identified in the genomes using ResFinder 4.0 [34]. The genetic comparison of the poxtA gene from different species was generated using Easyfig 2.1 [35]. Based on the draft genome sequences, a phylogenic tree was constructed for all sequenced poxtA-positive CoNS isolates by CSI Phylogeny 1.4 (https://cge.cbs.dtu.dk/services/CSIPhylogeny/ Accessed on: 29 January 2021), with the genome of GDY8P50P used as a reference. The tree was visualized using software Fig Tree 1.4.2. Single nucleotide polymorphism (SNP) divergence among various isolates carrying poxtA was calculated using snippy (https://www.github.com/heilaaks/snippy/ Accessed on: 29 January 2021).

4.5. Nucleotide Sequence Accession Numbers

The complete sequences of strains GDH8C97P, GDY8P33P, GDY8P50P, GDY8P58P, GDY8P60, GDY8P80P and GDY8P136P have been deposited in GenBank under accession numbers JADICE000000000, JADQVZ000000000, JADICF000000000, JADICG000000000, JADICH000000000, JADICJ000000000, respectively. The complete sequence of plasmid pY80 have been deposited in GenBank under accession numbers CP063444.

4.6. Ethical Considerations

The study was approved by the South China Agriculture University (SCAU) Animal Ethics Committee. The research was conducted in strict accordance with Section 20 of the Animal Diseases Act of 1984 (Act No 35 of 1984) and the Declaration of Helsinki, and was approved by the SCAU Institutional Animal Care and Use Committee.

5. Conclusions

In conclusion, this is the first study to report on the presence of the poxtA gene in livestock-derived *S. haemolyticus* and *S. saprophyticus*. The presence of IS1216-poxtA-IS1216 in *Staphylococcus* spp., *Enterococcus* spp. and *Pediococcus* spp. with different genetic and source backgrounds indicated an important role of IS1216 in the dissemination of poxtA. Moreover, the co-occurrence of poxtA with other antimicrobial and heavy metal resistance
genes on the transferable plasmids may lead to the co-selection of poxtA, contributing to its persistence and accelerating its dissemination even in the absence of direct selective pressure by the use of phenics, tetracyclines and oxazolidinones. Attention should be paid to the potential risks of the transfer of the plasmid-borne poxtA from enterococci and staphylococci to other Gram-positive bacteria. Therefore, routine surveillance for the spread of poxtA in different Gram-positive bacteria and the prudent use of antimicrobial agents in food-producing animals are urgently warranted.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/pathogens10050601/s1, Figure S1: Location of the poxtA-carrying plasmids in seven CoNS isolates and corresponding electrotransformants by S1-PFGE. Lanes M contain the XbaI pattern of *Salmonella braenderup* H9812 with the fragment sizes given in kilobases on the left-hand and right-hand sides; lanes 1 to 14 represent GDH8C97P, RN4220/pH97, GDY8P33P, RN4220/pY33, GDY8P50P, RN4220/pY50, GDY8P58P, RN4220/pY58, GDY8P60P, RN4220/pY60, GDY8P80P, RN4220/pY80, GDY8P136P and RN4220/pY136, respectively.

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**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the South China Agricultural University (SCAU) Animal Ethics Committee. The field sampling protocols, samples collected from livestock, strain isolation, and the research were conducted in strict accordance with Section 20 of the Animal Diseases Act of 1984 (Act No 35 of 1984), and were approved with the SCAU Institutional Animal Care and Use Committee.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

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